A PHARMACOKINETIC POPULATION MODEL FOR CYCLOSPORIN A IN RENAL TRANSPLANT RECIPIENTS

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Lê Thanh Trúc Vân

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ABBREVIATIONS

-2LL	minus two log likelihood, the objective function value in NONMEM
ADME	absorption, distribution, metabolism & elimination
ALAG	absorption lagtime, h
BSA	body surface area
BW	bodyweight
C_0	trough concentration
C_n	concentration "n" hours post-dose
CI	confidence interval
CL	clearance, L/h
CsA	Cyclosporin A
DV	dependent variable, observed concentration
ESRD	end stage renal disease
F	bioavailability
FDA	Food and Drug Administration
FO	first-order
FOCE	first-order conditional estimation
GOF	goodness of fit
*k*a	absorption rate constant, 1/h
ME	mean prediction error
MSE	mean squared prediction error
NONMEM	nonlinear mixed-effect modeling
NPD	naïve pooled data
OFV	objective function value
PD	pharmacodynamics
P-gp	P-glycoprotein
PK	pharmacokinetic
PPK	population pharmacokinetic
PRED	predicted concentration
Q	intercompartmental clearance, L/h
RES	residual
RMSE	root mean squared prediction error

RSE	relative standard errors	TX	transplantation
SPE	standardized prediction error	V_C	central volume, L
		V_d	volume of distribution, L
STS	standard two-stage	V_P	peripheral volume, L
TDM	therapeutic drug monitoring	WRES	weighted residual

ABSTRACT

Background: Cyclosporin A (CsA) has been a cornerstone of solid organ transplantation since its introduction to the market in the early 1980's, and is a major part of the success of immunosuppression in the clinical setting. CsA is like many other immunosuppressive drugs, and has a narrow therapeutic window and large inter-individual variability. Highly variable drug exposure is associated with a high risk of organ rejection, and side effects like nephrotoxicity, infection, hepatotoxicity, and cancer. Obtaining the optimal exposure of the drug will not only prevent acute rejection, but also prolong the survival of the grafts, the organs, and inevitably the patients.

The overall purpose was to develop a pharmacokinetic population model for further use in later studies to improve therapeutic drug monitoring of CsA in renal transplant patients. Specific goals for the thesis include testing different compartment models with different absorption and elimination profiles, screen for possible covariates that may improve the compartment model, and finally validate the model.

Methods: Data was gathered from three separate studies, previously performed by the Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo. 49 patients provided a total of 1027 plasma concentration samples and various patient demographics. By using the nonlinear mixed-effect modeling program NONMEM a pharmacokinetic population model was developed.

Results: A 2-compartment model with an absorption lagtime gave the best fit for the cyclosporin A data, with CL/F = 26.1 L/h, Q/F = 20.5 L/h, V_C/F = 77.7 L, V_P/F = 342 L, *k*a = 1.88 1/h, and ALAG = 0.452 h. Screening for covariates showed that age (years), body mass index (BMI, kg/m^2), creatinine clearance (ml/min.), gender, height (m), lean body mass (LBM, kg), steroid dose (mg), post-transplantation time (weeks), and weight (kg) were significant to varying degrees. The data-splitting as recommended by Food and Drug Administration (FDA) was employed as an internal validation of the model. The resulting objective function value (OFV) was very variable and clearly showed that the model has a serious lack of robustness. The difference between the maximum and minimum value were 253.09, which is significantly more than the maximum allowed value of < 3.84. Inclusion of

covariates that proved to be statistically significant may possibly have had their clinical significance overestimated. The criteria set up for inclusion of covariates in to the final model, will need revision to possibly remove some covariates and hopefully stabilize the model.

Conclusion: This model provides a good basis upon which a dosage regimen for cyclosporin A may be designed, though some further refinement may be needed to improve upon the models somewhat lack of robustness

1 INTRODUCTION

1.1 PHARMACOKINETICS

1.1.1 Introduction

Pharmacokinetics (PK) describes the relationship of drug concentrations attained in different regions of the body with time, during and after drug input. The drug level-time relationship is related to adjustable elements of dose, dosage form, frequency and route of administration. Simply put, PK is what the body does to the drug. It may be viewed separately from pharmacodynamics (PD), which covers the relationship between drug concentration and the magnitude of effects produced with time. In simple terms, PD is what the drug does to the body [1-3].

The drug concentrations needed for PK/PD evaluation are rarely measured at the site of action. Instead more accessible sites are used to assess exposure to drugs. The two most commonly sampled fluids are blood and urine [2, 3].

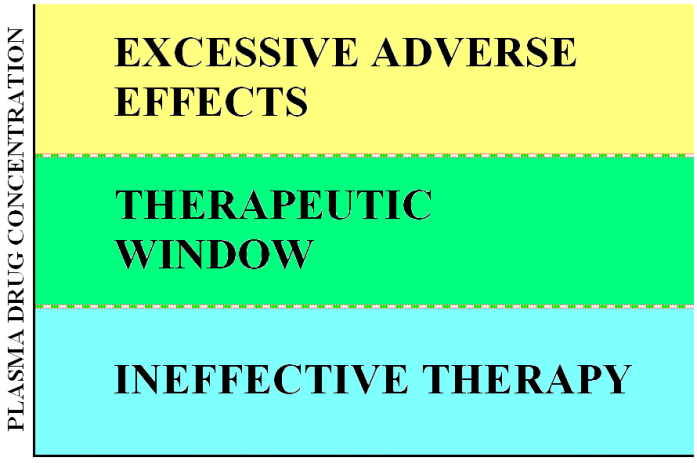


Figure 1. Therapeutic window

For every drug there exists an optimal range, where the drug gives its desired effects with acceptable side-effect intensity and therapy will be successful. Below this range the exposure to the drug is to low to give an adequate response, while too high an exposure will result in undesired adverse effects. This optimal range is called the therapeutic window. It is however important to point out that the limits for the therapeutic window is individual and will vary between patients [2, 3].

Often in PK/PD evaluation there will be a difference between expected values and the true outcome. These differences or variations can be attributed to inter-individual and residual variability. Inter-individual variability can be explained as when calculating parameter values based on past research and experience, the parameter values for a specific individual will differ from the expected values because of true biological variability between individuals. Inter-individual variability makes up a substantial part of the differences in drug response, usually reflected by the variety of drug strengths on the market. The residual variability is a grouping of several variations, including intra-individual variability, inter-occasion variability (day-to-day or week-to-week), and error in measurement, dosage and modeling. All this variability arises because the mathematical calculations that are used to estimate and predict the parameters are just an oversimplification of our reality. An increase in unexplained random variability produces uncertainty in predicting and controlling drug concentrations, and is of particular importance because it may decrease the efficacy and safety of the drug. It is important to keep in mind that the variability that applies to drug response will equally apply to adverse effects [2-6].

There are several factors that can influence the individual dose-concentration relationship, and this is recognized as variability in pharmacokinetic parameters. These factors include [2-5]:

- demographics; gender, body weight, body surface area (BSA), age, race etc.
- environmental factors; smoking, diet, exposure to pollutants etc.
- genetic phenotype of polymorphic cytochrome P450 isoforms that can affect the metabolism and clearance of drugs.
- interactions with other drugs, co-medication
- physiological factors; pregnancy and similar
- pathophysiological factors; renal impairment, hepatic impairment, CHD, other disease states
- other factors; circadian rhythm, adherence, food effect, timing of meals, physical activity, posture, stress

Variability, as properties of each individual that causes them to differ from an average individual, is called “fixed effects”. The other type of variability is called “random effects”, in the sense that they can not be predicted in advance. Random effects are comprised of inter-individual and residual variability, and quantify the amount of variability unexplained by the fixed effects [4, 7].

Studies using traditional pharmacokinetics do not deal with inter-individual variability, but rather estimates PK-averages. The inter-individual variability is viewed as a factor that needs to be overcome through rigorous and restrictive study design, which in turn makes the study design more complex. The patients that are selected for trial are standardized and homogenized. This creates artificial conditions which do not accurately represent the intended use of the drug [4, 5].

1.1.2 Population pharmacokinetics

The main goals of population pharmacokinetics (PPK) are to quantitatively assess the pharmacokinetic parameters, and the inter-individual and residual variability in drug absorption, distribution, metabolism and excretion (ADME). PPK highly contrasts with traditional pharmacokinetics. With PPK the goal is not to homogenize and standardize the patients, from which the data is gathered. PPK seeks to obtain all relevant information from the patients that are representative of those in whom the drug will be used clinically. This means that all sources of variability, including inter-individual, intra-individual, inter-occasion and unexplained variability, must be identified, explained and quantified. The clinical significance can be evaluated by identifying the measurable factors that is associated with change in the relationship between dose, concentration, response and pathophysiology, and the extent of these changes. Dosage may then be modified appropriately to maximize drug safety and efficacy [4-6].

With PPK it is possible to gain integrated information on PK from relatively sparse data, dense data or from a combination of both. Data can be divided into two groups: experimental data and observational (population) data. Experimental data are gathered through traditional studies, where there is controlled design and extensive blood sampling, i.e. dense data. Observational data on the other hand are gathered during routine clinical care or as a

supplement in a study designed and carried out for another purpose. These data are usually sparse, collected at various times, and unbalanced [5, 6].

PPK also seeks to quantify the distribution and spread of the variability. The measure of spread is termed variance, σ^2. By accounting for the variance in the model system, PPK can define the dispersion of drug exposure more precisely, thus lessening dispersion. By gaining more accurate and precise estimation of the PPK parameters and their variance, it is less probable that a concentration-driven toxicity will be encountered. This is because as the variance decreases, there will be fewer outliers [8].

PPK is most valuable in situations where the population in which the drug is intended is heterogeneous and when there is a narrow therapeutic window [6].

There are 3 interwoven steps in which population pharmacokinetics data analysis can be done [5, 6];

- Exploratory data analysis; where statistical and graphical techniques are used to uncover patterns and features in the population data.
- Pharmacokinetic population model development; where criteria and rationale for choice of model is set, and the steps taken to build the model is outlined
- Model validation; where the objective is to examine if the model describes the data in a good manner and produces a good fit.

1.1.3 Compartmental theory

Human anatomy and physiology is so complex that truly modelling how the body handles drugs is difficult. However it is often possible to simplify the body with regards to PK modelling into relatively few compartments [2, 3].

Compartments make up a basic model for drug absorption and disposition. There are two classes of compartments; transfer and chemical. Transfer compartments refer to different locations in the body. Chemical compartments are not locations, but refer to compartments that differ chemically. As in metabolism, the metabolite will be in a different chemical compartment than the drug itself. An often used representation of compartment models is the

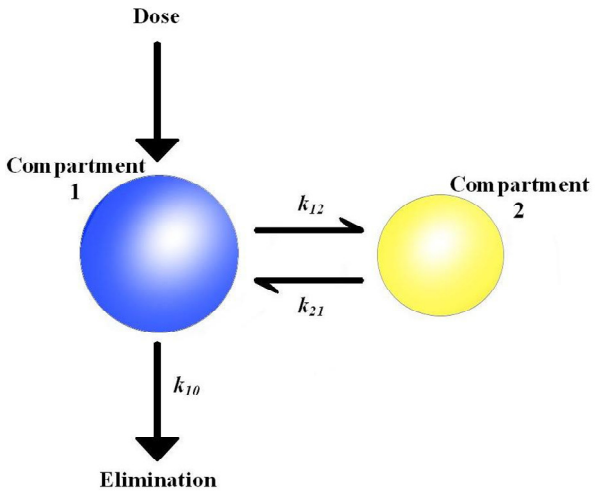


Figure 2. 2-compartment model with designated rate constants

2-compartment model as illustrated in figure 2. It shows drug administered and eliminated from the first compartment. Drug also distributes back and forth between the first and second compartment. It is important to point out that it is the plasma-concentration data that defines the model. Data is described using exponential terms, and the number of terms equals the number of compartments required. More complex models with bi-exponential and tri-exponential equations incorporate two and three compartments, respectively [2, 3].

1.2 POPULATION MODELING

1.2.1 Introduction

In PPK there are several parametric and nonparametric methods for estimating the parameters. Parametric methods have the ability to separate inter-individual, intra-individual and assay error. The weakness of this method is that it lacks mathematical consistency, and that it makes assumptions about the shape of the parameter distribution. Nonparametric methods on the other hand make no assumptions about the shape of the parameter distribution and can therefore detect possible subpopulations with other distributions. It is also mathematically consistent, but it does lack a feature to distinguish the various sources of variability. A few of the methods discussed here are the naïve approaches, the standard two-stage approaches and the mixed effects mode [9, 10].

1.2.2 Naïve pooled data approach

The naïve pooled data (NPD) approach as proposed by Sheiner and Beal [11], is a method in which all data gathered from every individual are recognized as data coming from only one unique individual. The NPD approach is a general approach that can incorporate routine pharmacokinetic data, nonstandard data and experimental data. This simple approach is widely applicable, though the drawback is that it may give misleading parameter estimates. Since all data is recognized as coming from only one individual, the reference to individual data is lost and all sources of inter-individual variability are lost. There will not be provided any estimates of the dispersion of parameters in the population, only mean parameter estimates. Another drawback that needs to be addressed with this approach is imbalance, which occurs when some individuals provide more observations than others [5, 9].

1.2.3 Standard two-stage approach

The standard two-stage (STS) approach is well known and widely used for more than 30 years, because of its simple method for pooling individual estimates of PK parameters. The downside is that it requires large numbers of plasma samples to be obtained from each participant in the studies, i.e. data rich situations. The minimum is at least one plasma concentration data point per parameter estimated. As the name indicates, the population parameter estimates are obtained in two stages. In the first stage, each subject's data are fitted separately, and from this the individual parameters are estimated using nonlinear regression. Also obtained from this step are the correlation and covariances between parameters in each patient. Then in the second stage, the parameters across the individuals are obtained. These include descriptive summary statistics, such as mean parameter estimates, variance and covariance. This approach usually gives unbiased mean parameter estimates, but have a tendency to overestimate the random effects. By weighting individual data according to quality and quantity, bias may be corrected and the STS approach can be improved [5, 6, 9, 10, 12].

1.2.4 The nonlinear mixed-effect model approach

Earlier attempts at modeling and estimating inter-individual pharmacokinetic parameter variability often neglected difficulties such as data imbalance, sparse data, and subject-specific dosing history. Sheiner et al. [7, 11, 13-16] were the first to attempt to include these difficulties arising from data of patients receiving therapy. This is said to be the first true population modeling program. The data per individual used in this approach is usually of a limited number, and collected under less restrictive conditions. This approach can handle as few as one sample per patient. Observational individual PK data tend to be sparse, unbalanced and fragmentary, and because the resulting data sets are too small to separately estimate the PK parameters for each subject, the STS approach must be excluded. Like the NPD approach, nonlinear mixed-effect modeling analyzes data from all the individuals simultaneously. The difference is that the inter-individual random effects structure is included during nonlinear mixed-effect modeling. The population mean values derives from fixed effects, and the variability within the population derives from random effects parameters. This method is well known to give PPK parameters with less bias (overestimation) than the other methods [5, 9, 10].

1.3 MAXIMUM LIKELIHOOD APPROACH

Estimation of the parameters in a model is most often done with the maximum likelihood approach, by minimizing the -2 log likelihood (-2LL)-function. -2 log likelihood is given by the following equation:

$$-2\log(L) = n\log(2\pi) + \sum_{i=1}^{n}\left(\log\left(\sigma_i^2\right) + \frac{\left(Y_i - \hat{Y}_i\right)^2}{\sigma_i^2}\right) \quad \textbf{Equation 1}$$

where Y is the measured observation, $\hat{Y}$ is the prediction of that observation by the model, and σ^2 is the variance of the model. The second part of the equation:

$\sum_{i=1}^{n}\left(\log\left(\sigma_i^2\right) + \frac{\left(Y_i - \hat{Y}_i\right)^2}{\sigma_i^2}\right)$ is sometimes called the “extended least squares” objective function, and from this the objective function value (OFV) can be obtained. To minimize

-2LL the effort needs to be directed towards this second part, since $n\log(2\pi)$ is a constant. Thus to minimize -2LL means that parameter estimates are chosen to maximize the probability of data under the model written as a function of the model parameters. This reveals the set of parameters that is more probable than any other set of parameters, as the best. The likelihood ratio test can therefore test for statistical significance between two or more models. Statistical significance is restricted under a given set of probability and degrees of freedom. It is however important to point out that the model with the lowest OFV is not necessarily the best model. Further, OFV cannot be used to compare different datasets. The choice of which model to be used must be justified by a better fit than the other models, especially when using more complex models. In a clinical setting the choice for model often falls on a fast and reliable model, even though it may not be statistically better [7, 9, 17].

1.4 NONMEM

1.4.1 Background

NONMEM was the first modeling program designed to analyze large amounts of PK data using nonlinear mixed-effect modeling. By utilizing first-order (FO) Taylor series expansion with respect to the random effect variables η_i and ε_{ij}, the program obtains linearity of the model in the random effects. From the following equation:

$$y_{ij} = f(\phi, x_{ij}, \eta_i) + \varepsilon_{ij} \quad \textbf{Equation 2}$$

the *j*-th measurement in the *i*-th subject of the population can be obtained. ϕ refers to the inter-individual parameter value estimates. η_i and ε_{ij} are independent, normally distributed with zero means and variances Ω and σ^2, respectively. By minimizing the term -2LL, the maximum estimates of population parameters θ, Ω and σ^2 can be obtained as follow:

$$-2LL = \sum_{i=1}^{N} (\log(\det(C_i)) + (y_i - E_i)^T C_i^{-1} (y_i - E_i)) \quad \textbf{Equation 3}$$

This is the most widely used approach in PPK, and is called the FO method [9].

NONMEM has two alternative estimation methods: the first-order conditional estimation (FOCE) method and the Laplacian method. FOCE as the name indicates uses Taylor series expansion with the parameter values distributed about conditional estimates (empirical Bayesian estimates) of the inter-individual random effects rather than zero. The Laplacian method use second-order expansion [9].

1.4.2 Modeling with NONMEM

NONMEM requires two specific files to be created by the user for modeling. One is the input file, which contains the data for describing the PK parameters, and the control file, which contains the model and parameter specifications [7].

Population modeling with NONMEM means that besides describing the PK parameters for the population, inter-individual and residual variability also needs to be described. An exponential statistical model for describing PK parameter inter-individual variability is expressed as:

$$P_{ij} = P_{TVj} \times Exp(\eta_{ij}) \quad \textbf{Equation 4}$$

In this equation P_{ij} is the *j*-th basic PK parameter for the *i*-th individual. P_{TVj} is the typical value of the *j*-th population parameter. The typical value of a parameter is the population estimate of that parameter, usually the mean. η_{ij} is a random variable for the *i*-th individual in the *j*-th parameter distributed with a mean of 0 and variance of ω_{ij} [18].

Residual variability can be described by a number of models: additive models, proportional (CCV; Constant Coefficient of Variation) models, exponential models, power function model, and combined additive and proportional model (slope-intercept model). Not all will be detailed in this thesis. More on this topic can be found in "NONMEM Workshop - Basic Concepts" by Shafer et al. [7].
The additive error model is described with the following equation:

$$Y = \hat{Y} + \varepsilon 1 \quad \textbf{Equation 5}$$

The additive error model is described with the following equation:

$$Y = \hat{Y} \times (1 + \varepsilon 1)$$ **Equation 6**

The combined model describes the residual variability with the following equation:

$$Y = \hat{Y} \times (1 + \varepsilon 1) + \varepsilon 2$$ **Equation 7**

In equation 4-6 Y is the observed concentration, $\hat{Y}$ is the predicted concentration, and the randomly distributed terms $\varepsilon 1$ and $\varepsilon 2$ have zero mean and variances $\sigma 1$ and $\sigma 2$, respectively [18, 19].

1.5 CYCLOSPORIN A

1.5.1 History of Cyclosporin A

Cyclosporin A (CsA) was first discovered through screening of lower fungus extracts. Active metabolites from the fungus *Cylindrocarpon Iucidum* booth showed mild antifungal activity and antibody depression in mice. Oral administration of the drug in mice significantly depressed the appearance of plaque-forming cells and produced an obvious dose-dependent inhibition of haemagglutinin. Skin graft rejection in mice was considerably delayed by CsA. Isolation of the active principle revealed a hydrophobic cyclic polypeptide of 11 amino acids (undecapeptide) with a molecular weight of 1202.06. Soil samples collected from Norway in March of 1970 showed that the fungus *Tolypocladium inflatum* also contained CsA. This fungus was originally classified as *Trichoderma polysporurn* (Link ex Pers.) Rifai. In 1972 CsA proved to have powerful immunosuppressive properties. Since then much research has been performed on the subject [20-23].

1.5.2 Applications and mechanism of action

Cyclosporin A has been a cornerstone of solid organ transplantation since its introduction to the market in the early 1980's, and is a major part of the success of immunosuppression in the clinical setting [18, 24]. CsA acts by forming a complex with the intracellular protein cyclophilin (an immunophilin), and this complex inhibits calcineurin. This will hinder the

activation of various transcription factors and ultimately lead to inhibition of interleukin-2 synthesis and decrease the proliferation and function of T cells. The mechanism of action of CsA is partially selective in that it suppresses T cells while to some extent spares B lymphocyte activity. CsA will therefore permit a better response to infections than other immunosuppressive drugs [1, 25].

1.5.3 Known problems with Cyclosporin A

When administering immunosuppressive drugs to organ transplant recipients, it is crucial to obtain the optimal exposure of the drug. This will not only prevent acute rejection, but also prolong the survival of the grafts, the organs, and inevitably the patients, simultaneously by also minimizing the side-effects. Cyclosporin A is like many other immunosuppressive drugs, and has a narrow therapeutic window and large inter-individual variability. This applies particularly after oral administration, to which observations have shown great variability within the first 4 hours. With concentration levels below the therapeutic window there is a high risk of organ rejection, while concentration levels above the therapeutic window is associated with side effects like nephrotoxicity, infection, hepatotoxicity, and cancer. Highly variable drug exposure may over time lead to chronic nephropathy, due to renal vasoconstriction. Glomerular filtration rate (GFR, ml/min) will decrease and cause hypertension, which will alter renal prostaglandin biosynthesis. It may necessitate the withdrawal of CsA. There are also a number of drugs that are known to interact with CsA, and may either lead to a decrease or an increase in CsA blood levels. Due to these particular problems, there are few PPK studies dealing with CsA modeling, but on the other hand it is for such a drug PK really can make a difference if used clinically. Especially the absorption profile of CsA makes PK modeling very difficult [1, 18, 25-31].

1.5.4 ADME

CsA may be administered by intravenous infusion or orally, for which the peak plasma concentration is obtained after ~3-4 hours. The absorption profile of CsA is characterized as flat and delayed, with a correlation between delay and peak width [1, 27].

Bioavailability of CsA depends highly on the population studied, but will normally range from 30-60 % [24, 29, 32].

Highly lipophilic drugs such as CsA bind to tissue to a great degree and would therefore have a volume of distribution that exceeds the 42 L that make up the total volume of water in the body. Within whole-blood CsA will distribute highly to erythrocytes, and in a lesser degree to lymphocytes and granulocytes. CsA has a large V_d even though it is highly bound to blood cells and plasma lipoproteins [3, 24, 32].

Metabolism of CsA to its approximately 30 metabolites is extensively by the cytochrome P450 system, in particular CYP3A4 in majority and also CYP3A5. It is also a subject and inhibitor for the ATP-binding cassette transporter protein, P-glycoprotein (P-gp, mdr-1 / ABCB1). The metabolic pathways of CsA are made up of Phase I biotransformation by CYP enzymes, which include oxidation, dealkylation, and hydroxylation. CYP3A and P-gp work in concert to hinder CsA access to the systemic blood circulation. P-gp by counter transporting CsA out of the enterocyte, i.e. efflux, and back into the gut lumen, makes CsA available once again for metabolism by the CYP enzymes. This cycle greatly enhances drug metabolism. As these systems are present in a large degree in the intestines and the liver, CsA is therefore subject to a large first pass effect, and hence have low oral bioavailability. Genotypical differences in CYP3A5 protein expression have proven to cause notable variations in CsA PK. Less consistent is the association between polymorphism of the mdr-1 gene and CsA PK. Mutation in the mdr-1 gene may cause lower P-gp levels, which may decrease the dose requirement for CsA [3, 24, 33-38].

The biliary system is responsible for most of the elimination of CsA, and only 6 % of the metabolites are excreted renally. It is worth mentioning that the absorption of CsA requires an adequate flow of bile, so that CsA is also a part of enterohepatic recycling [24, 29, 30, 39].

Half life of CsA varies to a great extent, with approximately 6.3 hours in healthy individual and to 20.4 hours in patients with serious liver complications [32].

Even though there are much literature on the matter of the PK and PD of CsA, there are still difficulties in predicting the disposition in specific individuals. The need for a population approach is evident when reviewing the many causes for variation [18].

1.5.5 The need for therapeutic drug monitoring

The causes for pharmacokinetic variability of CsA are complex and difficult to predict, which usually renders therapeutic drug monitoring (TDM) mandatory to maximize the immunosuppressive effects of CsA. The index most closely linked to the therapeutic effect and also the toxic effects, is thought to be the inter-dose area under the plasma concentration curve from 0 to 12 hours (AUC_{0-12}). This monitoring approach is both time consuming and expensive, and rarely done in routine clinical practice. However, the optimal method for TDM is still debated, and blood level measurements are often performed either at trough level (C_0), and/or 2 hours after dosing (C_2). The downside to measuring single samples is of course less accurate drug exposure predictions. C_2 measurements are believed to be better associated with AUC_{0-4}, which is also considered a marker for toxic effects [24-28, 31, 40, 41].

1.5.6 PK models of Cyclosporin A in litterature

Findings in literature show that there have been many attempts to model the PK of CsA, and all have come to a different conclusion as of what is the PK population model that best describes this. Both 1- and 2-compartments are used in these different models, and different absorption methods have been used. For some models the Erlang distribution gave a good fit, while others used an absorption lagtime. In addition each study tested a wide range of covariates on their model [18, 28, 29, 34, 35, 41, 42]. It was therefore necessary to try out many different models in our search.

1.6 GOALS

The goal of this thesis is to make a PK population model for CsA, by using data gathered from previous clinical trials performed at the Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo. The final model is then to be used in an add-on study to compare the effectiveness of TDM of CsA using NONMEM, against TDM using traditional clinical C_2 monitoring. This add-on study will provide a real-time data assembly

and evaluation that can provide drug exposure safety monitoring. Results from this study will not be presented in this thesis.

Specific goals for the thesis include testing different compartment models with different absorption and elimination profiles, screen for possible clinically relevant covariates that may improve the compartment model, and apply internal validation as well as external validation methods.

2 METHODS AND MATERIALS

2.1 STUDY DESIGN AND POPULATION

Data were obtained from 49 patients who received renal transplantation at Rikshospitalet University Hospital HF, Oslo, Norway. CsA (Sandimmun Neoral®, Novartis Pharmaceuticals Corporation, Switzerland) was administered orally twice daily in soft gelatin capsule formulation, along side other routine protocol medication.

The data were obtained from three separate prospective clinical trials, so there were variations in the amount of information gathered. The medical records contained date, time, transplantation date, CsA dosage, CsA blood concentration, gender, age, weight, height, serum creatinine, urea, and concurrent medication. A full PK population sampling design was used to allow blood samples to be drawn at various times. This allowed estimation of PK parameters and explanation of variability [6]. A total of 1027 drug concentration monitoring data points was gathered.

Patients 1-20 were from the SUPER-CsA study [43]. This was a single centre prospective pilot study following patients from 0 to 17 weeks post-transplantation, with measurements made sporadically at trough level (C_0) and 2 hours after CsA administration (C_2). Nine of these patients (patients 7-12, 14 and 18-19) had a 12-hours pharmacokinetic profiling done once during the study period. During the 12-hours, measurements of whole-blood CsA concentrations were made at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after CsA administration. All of the data gathered from the patients were included in the model. Analyses of the whole-blood CsA concentrations were performed using the CEDIA Cyclosporine PLUS assay (Cloned Enzyme Donor Immunoassay; Microgenics Corporation, Fremont, CA) as described by Falck et al. [43], except the 12-hours PK-profiling whole-blood samples which were analyzed for CsA concentrations with a validated LC-MS/MS method [44, 45].

Patients 30-37 were from the MIMPARA study [45], which is an interaction study between Cinacalcet® and immunosuppressive drugs. 13 whole-blood plasma concentration

measurements (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 hours after administration) were performed during a 12-hours pharmacokinetic profiling between 3-10.4 weeks after renal transplantation. Only CsA data from before Cinacalcet® administration were used in this model development. Whole-blood samples (heparine vacutainers) were immediately frozen and stored at −30◦C until analyzed for CsA concentrations with a validated HPLC-MS/MS method [45].

The remaining patients (51-65 and 67-72) were from a CsA study performed to find any possible effect of age on the PK of CsA [44]. These patients also had a 12-hours PK-profiling performed between 2-9 weeks after renal transplantation. Whole-blood CsA concentration were measured at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after administration of CsA. The whole-blood samples were analyzed for concentrations of CsA using a validated LC-MS/MS method [44].

Whole-blood samples for the 12-hours PK-profiling were analyzed at both the study center, Rikshospitalet University Hospital HF, and by the Department of Pharmaceutical Biosciences. While whole-blood samples taken sporadically were only analyzed by Rikshospitalet University Hospital HF. Analysis results showed that there was significant inter-laboratory variability. This may be the result of the different analysis methods. All CsA concentrations analyzed by the Department of Pharmaceutical Biosciences were therefore adjusted to the correct concentration, as defined by Rikshospitalet University Hospital HF, with the following equation:

$$RH = DPB \times 0.88 \quad \textbf{Equation 8}$$

where RH is the adjusted concentration according to Rikshospitalet University Hospital HF, and DPB is the concentration obtained from analysis performed by the Department of Pharmaceutical Biosciences. This equation was obtained from correlation of concentrations measured at both laboratories in the three studies. [43-45]

As data was gathered at various times, it was in a sense no missing data points to speak of. There was therefore no need to impute any data points that might have influenced the bias.

The exact time of each blood sample was written down, except for some of the samples taken sporadically as mentioned above. All data points were included in the input file for

NONMEM. Sporadic measurements were coded for C_0 as 06.00 hours for morning doses and 20.00 hours evening doses, while C_2 was coded as 08.00 hours for morning doses and 22.00 hours evening doses.

2.1.1 Cofactors influencing Cyclosporin A

Before choosing which cofactors that is relevant and that need to be studied, it is important to distinguish between clinically relevant and statistically significant covariates. There needs to be a rationale behind the choices that are made [5].

Cofactors chosen to be studied were based on findings in literature and clinical findings. Weight (kg), creatinine clearance (ml/min.), type 1 diabetes (1=non-diabetic; 2=type 1 diabetes), age (years), gender (1=male; 2=female), height (m), post-transplantation time (weeks), steroid dose (mg), body mass index (BMI) (kg/m^2), CYP 3A5 genotype (1=*1/*3; 3=*3/*3), and lean body mass (kg), were screened to uncover any possible influence on the proposed PK population model [26-28, 35, 40, 46, 47]. Lean body mass was calculated from the height and weight of the patients with the following equations [48]:

$$\textit{Male;} \qquad L.B.M. = 0.32810\,W + 0.33929\,H - 29.5336 \quad \textbf{Equation 9}$$

$$\textit{Female;} \qquad L.B.M. = 0.29569\,W + 0.41813\,H - 43.2933 \quad \textbf{Equation 10}$$

Creatinine clearance was calculated using the Nankivell equation [49]:

$$\frac{6700}{Serum\ creatinine\ (\mu mol/L)} + \frac{Weight\ (kg)}{4} - \frac{Urea\ (mmol/L)}{2} - \frac{100}{Height\ (m)^2} + \left[35\ (female)\ or\ 25\ (male)\right](ml/mi.n) \quad \textbf{Equation 11}$$

Saint-Marcoux et al. [26] encoded post-transplantation time as a factor for variability in their population model for CsA. They divided the time frame into three periods, < 2 weeks, between 2 weeks and three months, and > 3 months. In our model we chose to code post-transplantation time as a continuous covariate with a linear and proportional model and also as a categorized continuous covariate, divided into < 2 weeks and ≥ 2 weeks.

Table 1. Patient demographics

	Value	Mean	Range	
Total number of patients	49			
Number of patients with type 1 diabetes	9			
Age (years)		55.0	21.0	78.6
Weight (kg)		78.5	49.0	106.5
Height (m)		1.76	1.53	1.92
Body mass index (kg/m^2)		24.9	18.9	35.2
Lean body mass (kg)*		55.7	36.4	66.5
Gender;				
Male	33			
Female	16			
CYP 3A5 genotype;				
***1/*3**	6			
***3/*3**	43			
Post-transplantation time (weeks)		5.1	0.0	17.0
Total daily steroid dose (mg)		23.2	0.0	80.0
Creatinine clearance (ml/min.)*^{2}		71.35	6.78	162.50
Cyclosporine;				
Dose (mg/12 hours)		216	25	600
Observed whole-blood concentration (ng/ml)		1056	30	3240
Total number of samples	1027			
Average number of samples per patient	21.0			

* calculated using equation 9 and 10
*^{2} calculated using the Nankivell formula (equation 11)

A factor that might influence the overall result of model building is the fact that all the patients received their steroid dose in the form of orally administered prednisolone, with the exception of patient no. 2 that started out on oral prednisolone, and later switched over to i.v. Solu-Medrol®. The significance of this variable was not tested in this model.

2.2 BUILDING THE POPULATION MODEL

The pharmacokinetic population modeling was done with NONMEM (version VI; GloboMax LLC, Hanover, MD, USA) and the graphical diagnostics was obtained by using the program R (http://www.r-project.org/) and Microsoft Office Excel 2003©. The model building process is made up of different steps. The first step before building the population model was to compile all of the data available into the input file for NONMEM to read

(Appendix 7.1). Data from patient charts was inserted into an input file and double checked against information available at the study center to detect and correct any possible errors.

The second step was to create and test control files with different compartment models with first-order elimination, and include first- or zero-order absorption, with or with out absorption lagtime. The number of compartments ranged from one to three. Criteria for choice of model to be used in the next step included the model that gave the best statistical result, i.e. the lowest OFV value, had a short run time, and also the model that gave the best stability during testing [7, 19].

The parameters used to describe the compartment models includes clearance (CL), central volume (V_C), peripheral volumes (V_{Pn}), intercompartmental clearances (Q_n), absorption rate constant (*k*a), rate constant between the central and peripheral compartments (k_{CP}, k_{PC}), and absorption lagtime (ALAG) [19]. Also tested was the Erlang distribution, because of the flexibility in modeling flat or delayed absorption profiles. The Erlang distribution is a special form of gamma distribution and describes a right skewed absorption lag. More specifically the Erlang distribution is an analytical solution for a number of "n" compartments linked together with the same transfer rate constant, k_{tr}. These theoretical Erlang compartments are positioned between the depot and the central compartments of the population model [26, 27].

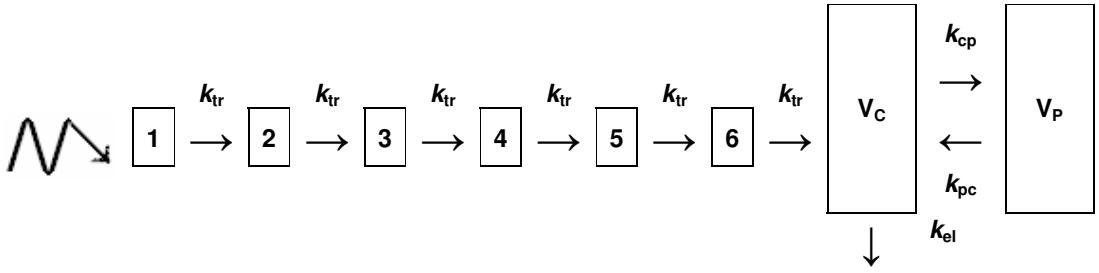


Figure 3. This illustrates a 2-compartment model with an Erlang distribution [27].

The rate of exit, $f(time)$, from the Erlang compartments can be described by the following equation:

$$f(time) = \frac{k_{tr}^{n} \times t^{n-1} \exp(-k_{tr} \times t)}{(n-1)!} \quad \textbf{Equation 12}$$

Determining the number of sequential Erlang compartments was done by using the criteria that the number of compartments was increased until no improvement or deterioration of the model performance could be seen. The same number of Erlang compartments, n, is then set to all the patients in the data set [26]. k_{tr} would in an Erlang model replace *k*a of ordinary compartment models.

Unlike absorption lagtime which just gives a delay in absorption, the Erlang distribution models an increasing transition for the absorption. This may create a more realistic model of the absorption process for drugs such as CsA.

During the model building process the error model used to describe the residual variability was the combined additive and proportional model. While an exponential error model was used to describe the inter-individual variability. These choices for error models were based previous experience and findings in literature [27, 50].

NONMEM provides several estimation methods, and the ones utilized in during model building were FO and FOCE [28].

The NONMEM program relies on the estimation of parameter through statistical significance of the maximum likelihood approach. By comparing the resulting OFV the different models can be compared. The criterion set for improvement in fit is a drop in OFV > 3.84 [7].

2.3 ANALYZING FOR COVARIATES

2.3.1 Introduction to methods used

The third step when building a PPK model is to establish the relationship between the model parameters and the covariates. The covariates are patient specific variables that can explain parameter variability. Covariates are divided into continuous covariates and categorical covariates. In modeling continuous covariates are usually centered in the normal population model, while categorical covariates are handled by one or more IF / ELSE statements [51, 52].

The covariate model is built in a stepwise manner using forward inclusion - backward deletion. In the first step, the start model is the basic model without covariates. To this start model, all possible parameter-covariate combinations are tested in turn. The number of these combinations is often large, for example 11 covariates with 6 parameters would give a total of 66 combinations. These combinations would then be modeled with different covariates equations. If on average there are 5 equations for each combination, it would result in 330 different control files that would need to be run through NONMEM. It is safe to say this is a time consuming process [18, 51-54].

The covariates were tested with the following equations [7]:

Linear model:

$$TV_{pop} = \theta_p + \theta_1 \times covariate\ value \quad \textbf{Equation 13}$$

$$TV_{pop} = \theta_p - \theta_1 \times covariate\ \ value \quad \textbf{Equation 14}$$

Proportional model:

$$TV_{pop} = \theta_p \times covariate\ \ value \quad \textbf{Equation 15}$$

$$TV_{pop} = \theta_p / covariate\ \ value \quad \textbf{Equation 16}$$

where TV_{pop} is the typical value of the population estimate, θ_p is the individual parameter estimate, and θ_1 is the factor contributed by the covariate. In addition, the continuous covariates in the linear model were centered to their mean value [7].

Linear model:

$$TV_{pop} = \theta_p + \theta_1 \times (covariate\ \ value - mean\ covariate\ value) \quad \textbf{Equation 17}$$

$$TV_{pop} = \theta_p - \theta_1 \times (covariate\ \ value - mean\ covariate\ value) \quad \textbf{Equation 18}$$

An example is given with the following equation:

$$V2/F = \theta_2 + \theta_7 \times (BW - 78.46) \quad \textbf{Equation 19}$$

This equation represents the relationship between *V2/F* and bodyweight (BW) in kg. *V2/F* is the population mean value of *V2/F* in liters, θ_2 is the *V2/F* value of a median patient weighing 78.4 kg, and θ_7 is the difference in *V2/F* per kg BW, with the population average taken into consideration [26].

The categorical covariates were handled by the following IF / ELSE statements [7]:

Type 1;

$$\begin{aligned} &IF\ ([Covariate] = or \le [value])\ THEN \\ &TV[Parameter] = \theta_p \times \theta_1 \\ &ELSE \\ &TV[Parameter] = \theta_p \times \theta_2 \\ &ENDIF \end{aligned}$$ **Equation 20**

or

Type 2;

$$\begin{aligned} &IF\ ([Covariate] = or \le [value])\ THEN \\ &TV[Parameter] = \theta_q \\ &ELSE \\ &TV[Parameter] = \theta_r \\ &ENDIF \end{aligned}$$ **Equation 21**

In addition to these models, the following model was decided to be tested:

$$TV_{pop} = \theta_p + \theta_1 \times (1 + covariate\ value/100)$$ **Equation 22**

$$TV_{pop} = \theta_p - \theta_1 \times (1 + covariate\ value/100)$$ **Equation 23**

The inclusion criteria are based on the likelihood ratio test, and rely on improvement in fit. During inclusion of covariates, a reduction in OFV > 3.84 is considered significant at $p < 0.05$, and a reduction > 6.63 is considered significant at $p < 0.01$. Covariate combinations that give a significant drop in OFV are included in the model for the next step. The next step involves including all significant covariates into one model, the full model. From this full model, one covariate is removed at a time. This process is called backwards deletion. A

stricter criterion is used during backwards deletion, and requires significance at $p < 0.05$, i.e. an increase in OFV of > 6.63. An increase in OFV > 10.9 is considered significant at $p < 0.01$. When no more covariates can be excluded from the model according to the criteria, then the final model is established. The final model would then only include the covariates that proved to be statistically significant [26, 30, 51, 52, 54]. Covariates were assumed to be constant within an individual for the sake of simplicity [4].

To perform backwards deletion where one covariate is removed at a time from the full model, THETA-value of the linear models are fixed at 0, THETA-value of the type 1 IF / ELSE statements (Equation 16) are fixed at 1, and for the other models the covariate was removed entirely from the control file.

2.4 CRITERIA FOR CHOICE OF MODEL

The decision for what would be the final structural model was made considering 1) the objective function value (OFV), 2) the residual variability, 3) correlation and regression analysis between predicted concentration (IPRE/PRED) and observed concentration (DV), 4) the aspect of the weighted residual (WRES, residuals weighted by the standard deviation) plots, 5) graphical analysis of concentration-time curves, and 6) clinical applicability [26, 28, 30].

2.5 VALIDATING THE MODEL

2.5.1 Internal validation

2.5.1.1 *Examining the predictive performance of the population model*

A PK population model needs to show high goodness of fit, good stability, reliability and predictive performance. Following FDA recommendations [6] data-splitting was used to randomly divide the full dataset into 10 subsets, each containing ~ 90 % of the patient data. The data were randomized into 10 subgroups by using a combination of the “Random

Sequence Generator" and the "Random Integer Generator" on the website http://www.random.org [55].

Table 2. Subgroups after data-splitting

Subgroup	Patients excluded				
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
A	2	8	32	54	33
B	3	17	60	61	53
C	11	4	34	72	67
D	13	10	36	69	57
E	12	5	55	68	37
F	15	7	59	52	
G	20	19	65	30	64
H	18	6	70	71	58
I	14	1	31	51	56
J	9	16	63	62	35

The subsets with the included patients were then analyzed individually using the final model. Parameter estimates obtained were then compared to those obtained from the full data set. The parameter estimates from each of the subsets were also used to predict the CsA concentrations of the data subsets containing the remaining ~10 % of the patients. This was done by removing the observed concentrations from the ~10 % data subset and running NONMEM with the commands, "MAXEVAL=0" and "posthoc" in the $ESTIMATION step. Four different data sets were created for each subgroup. For the first data set all the observed concentrations were included, for the second data set no observations were included, for the third data set only the first observed concentration was included, and for the last data set the first and second observed concentration was included. In addition the objective function value was calculated when applying the parameters obtained from the 10 subsets with included patients on to the full data set. The OFVs were compared to that obtained from the final model. The intent was to examine the validity of the parameter estimates, the robustness of the final model, and to evaluate the predictive performance of the final model [6, 18, 26, 28, 50, 56, 57].

To calculate the difference between the observed and the model-predicted concentrations, the prediction errors on concentration was used. Mean prediction error (ME, ng/ml) was calculated as a measure of bias, and mean squared prediction error (MSE, $(ng/ml)^2$) and root mean squared error (RMSE, ng/ml) was calculated to assess the precision. Predictive performance was also assessed by standardized prediction error (SPE).

$$ME = \frac{1}{n}\sum_{i=1}^{n}(C_{Pred} - C_{Obs}) \quad \textbf{Equation 24}$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(C_{Pred} - C_{Obs})^2 \quad \textbf{Equation 25}$$

$$RMSE = \frac{1}{n}\sum_{i=1}^{n}\sqrt{(C_{Pred} - C_{Obs})^2} \quad \textbf{Equation 26}$$

$$SPE = \frac{C_{Obs} - C_{Pred}}{SD_{Cpred}} \quad \textbf{Equation 27}$$

where C_{Obs} is the observed concentration and C_{Pred} is the predicted concentration. SD_{Cpred} is the standard deviation of the predicted concentration and the total number of observations in the validation group is given by the number *n* [5, 18, 51].

Besides data-splitting there is another technique to internally validate a model, which is called resampling. There are two ways to perform resampling [5, 6]:

- Cross-validation: a repeated data-splitting validation, where fewer data points are discarded during the estimation process.
- Bootstrapping: using the entire dataset, it is useful for datasets with limited sizes [57].

These techniques were not utilized and will therefore not be discussed further.

2.5.2 Confidence interval

Confidence intervals (standard errors) for the parameters can be estimated using a nonparametric technique called “The Jackknife”. The procedure entails excluding parts of

the data one by one, in this case one patient is excluded from the dataset at a time, which produces a total of 49 Jackknife data input files. Following this, each Jackknife data set is tested with the final model. Parameter estimates from these NONMEM runs are used to calculate the confidence interval. When estimating standard errors and bias, bootstrapping might perform better, but the Jackknife has the advantage of requiring less computation [5, 53, 58, 59].

2.5.3 External validation

The external validation process is where we apply the developed model on to a new set of data from another study [57]. The external data not used to develop the model will provide estimation of the *actual prediction error*, as opposed to the *apparent prediction error* obtained when estimation is done through internal data-splitting [60].

The external data set comprised of 10 anonymous patients who received renal transplantation at Rikshospitalet University Hospital HF, Oslo, Norway. All 10 patients were given routine treatment according to protocol, with C_0 and C_2 sampling performed according to Rikshospitalet University Hospital HF's protocol. This provided 215 CsA concentration measurements. The whole-blood samples were collected between 0-12 weeks after renal transplantation. Patient demographics relevant to the model were also provided in the patients charts. Whole-blood samples were analyzed for CsA concentrations using the CEDIA Cyclosporine PLUS assay (Cloned Enzyme Donor Immunoassay; Microgenics Corporation, Fremont, CA) as described by Falck et al. [43]

Data sets were created with all the observed concentrations, no observed concentrations, the first observed concentration, observed concentrations from the first week or observed concentrations from the second week. The individual predicted concentrations from these five data sets were compared to the actual observed concentrations. The same prediction errors used for predictive performance were calculated (equation 24-27).

Table 3. External validation patients demographics

	Value	Mean	Range	
Total number of patients	10			
Number of patients with type 1 diabetes	1			
Age (years)		59.2	35.0	77.0
Weight (kg)		67.4	40.0	102.0
Height (m)		1.68	1.53	1.87
Body mass index (kg/m^2)		23.0	16.2	30.5
Lean body mass (kg)*		50.3	36.9	66.0
Gender;				
Male	4			
Female	6			
CYP 3A5 genotype;				
***1/*3**	NA			
***3/*3**	NA			
Post-transplantation time (weeks)		4.7	0.0	12.0
Total daily steroid dose (mg)		17.1	10.0	50.0
Creatinine clearance (ml/min.)*^{2}		62.78	4.83	125.29
Cyclosporine;				
Dose (mg/12 hours)		154	50	400
Observed whole-blood concentration (ng/ml)		1098	85	2595
Total number of samples	215			
Average number of samples per patient	21.5			

* calculated using equation 9 and 10
*^{2} calculated using the Nankivell formula (equation 11)

The appropriateness of the final model will also be reviewed by comparing dosage strategies. Real-time data assembly and evaluation from an add-on study gives the opportunity to compare the NONMEM based TDM against clinical TDM. The results from this add-on study will start during the 2nd quarter of 2008 and will therefore not be presented in this thesis.

3 RESULTS

3.1 MODEL BUILDING RESULTS

Model development was a time consuming task were many different models had to be screened to evaluate the appropriateness of the model compared to the CsA data provided. The screening process was performed while keeping in mind the criteria mentioned in paragraph 2.4. Inclusion of the Erlang distribution proved to be time consuming and was therefore only tested on the most promising model, which was the 2-compartment model.

Table 4. Results from model screening

Compartment model	Model specifications	Objective function value	Residual variability		Run time (hh:mm:ss)
			Proportional (%)	Additive (ng/ml)	
1-compartment	1. order absorption	13035.80	36.19	0.01	00:01:21
1-compartment	1. order absorption w/ lagtime	12807.03	31.94	2.69	00:04:40
2-compartment	1. order absorption	12805.39	31.40	6.34	00:11:32
2-compartment	1. order absorption w/ lagtime	12403.00	24.86	4.83	00:17:02
2-compartment	1. order absorption w/ lagtime & covariates	12088.5	19.18	5.38	00:24:36
2-compartment	0. order Absorption w/ lagtime	12954.06	34.93	0.98	00:10:24
2-compartment	1 Erlang compartment	12614.71	27.46	27.42	04:46:17
2-compartment	2 Erlang compartment	12512.24	25.86	24.76	17:03:55
2-compartment	3 Erlang compartment	12469.43	25.24	21.12	10:34:43
2-compartment	4 Erlang compartment	12439.46	24.25	29.19	26:28:56
2-compartment	5 Erlang compartment	12816.19	31.94	0.37	03:36:47
2-compartment	6 Erlang compartment	12430.74	24.23	23.04	83:20:34
2-compartment	7 Erlang compartment	12434.28	24.29	23.24	98:13:25
3-compartment	1. order absorption	12776.76	31.62	3.16	00:51:24
3-compartment	1. order absorption w/ lagtime	12457.45	25.16	35.07	01:10:49

Applying elaborate functions to the control file further complicates the model, which can be seen by the steady increase in run time when functions such as lagtime, covariates and Erlang distribution is added (Table 4). The upside of making an elaborate model is the fact that it better describes the data and to a certain point decreases the OFV.

Table 5. Parameter estimates of the models during preliminary screening

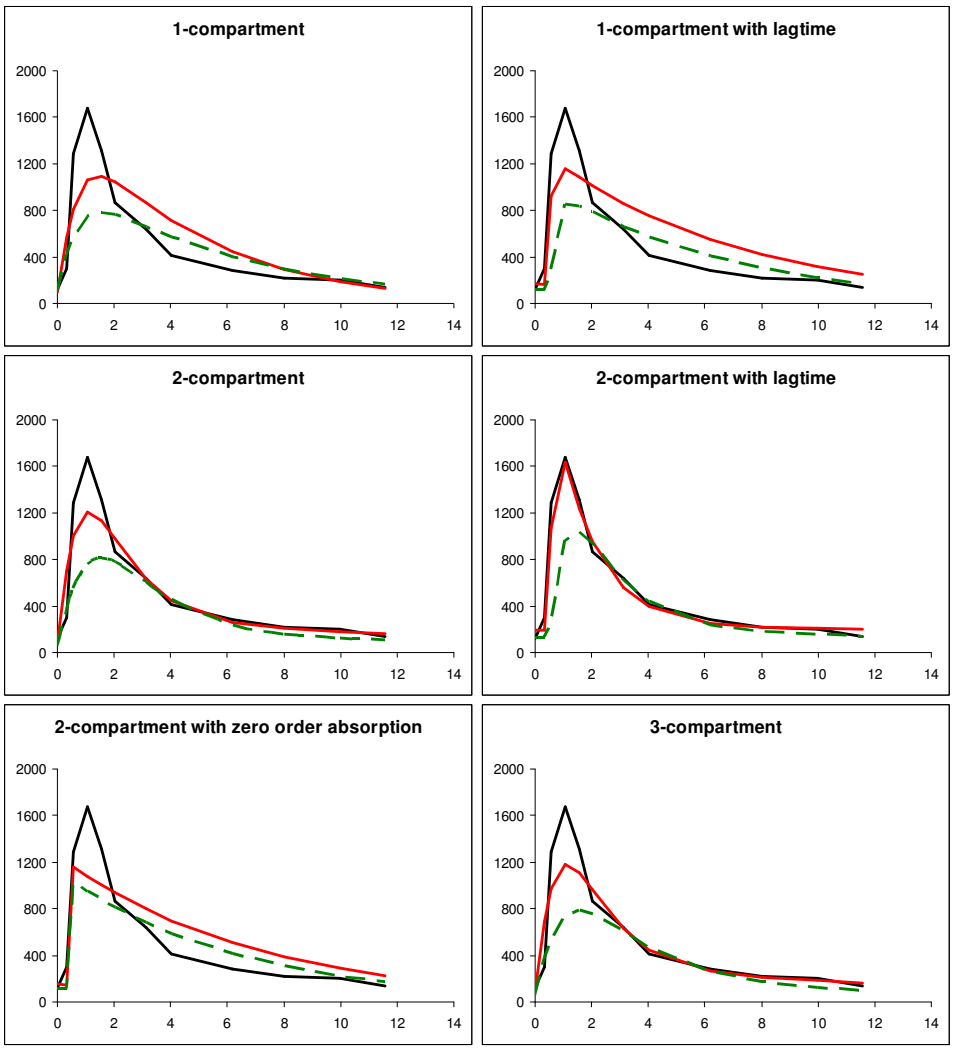
Compartment model	Model specifications	CL/F	Q/F		V/F			*ka* ; *k*tr	ALAG
			1	2	C	P1	P2		
1-compartment	1. order absorption	23.4	-	-	138	-	-	1.6	-
1-compartment	1. order absorption w/ lagtime	23.2	-	-	145	-	-	4.79	0.454
2-compartment	1. order absorption	23.3	18.3	-	54.8	277	-	0.615	-
2-compartment	1. order absorption w/ lagtime	26.6	19.2	-	80.3	343	-	1.86	0.452
2-compartment	1. order absorption w/ lagtime & covariates	26.1	20.5	-	77.7	342	-	1.88	0.452
2-compartment	0. order absorption w/ lagtime	22.1	186	-	1.15	120	-	0.32	0.33
2-compartment	1 Erlang compartment	24	25.2	-	62	331	-	1.49	-
2-compartment	2 Erlang compartment	24.8	29.9	-	66.5	289	-	2.5	-
2-compartment	3 Erlang compartment	24.7	27.4	-	82	286	-	3.93	-
2-compartment	4 Erlang compartment	23.6	24	-	91.7	389	-	5.58	-
2-compartment	5 Erlang compartment	23.6	23.1	-	145	215	-	8.23	-
2-compartment	6 Erlang compartment	23.9	24.5	-	95.2	377	-	8.14	-
2-compartment	7 Erlang compartment	23.8	23.9	-	96.5	366	-	9.59	-
3-compartment	1. order absorption	21.8	10.1	11	81.1	25.2	498	0.897	-
3-compartment	1. order absorption w/ lagtime	23.7	10.1	21.3	50.6	54.6	187	1.2	0.447

Abbreviations; CL = clearance, Q_1 = intercompartmental clearance for peripheral compartment 1, Q_2/F = intercompartmental clearance for peripheral compartment 2, V_C = central volume, V_{P1} = peripheral volume 1, V_{P2} = peripheral volume 2, *k*a = absorption rate constant, ktr = Erlang transfer rate constant, ALAG = absorption lagtime, F = bioavailability

Parameter values estimated from the different compartment models show consistency and are comparable to parameter values listed in other studies, such as those of Bourgoin et al.[41], Fanta et al. [29], Hesselink et al. [34], Irtan et al. [28] and Wu et al.[18].

3.1.1 Graphical comparison of the models

Patient no. 70 was chosen to demonstrate the graphical process of model building. The 12-hours PK-profiling was plotted and compared between the different PK population models. Visual analysis based on structure and fit of the plot was included in the decision for model development. Improvement of individual predicted concentrations was weighted more than improvement of population predicted concentration at this point in model development.



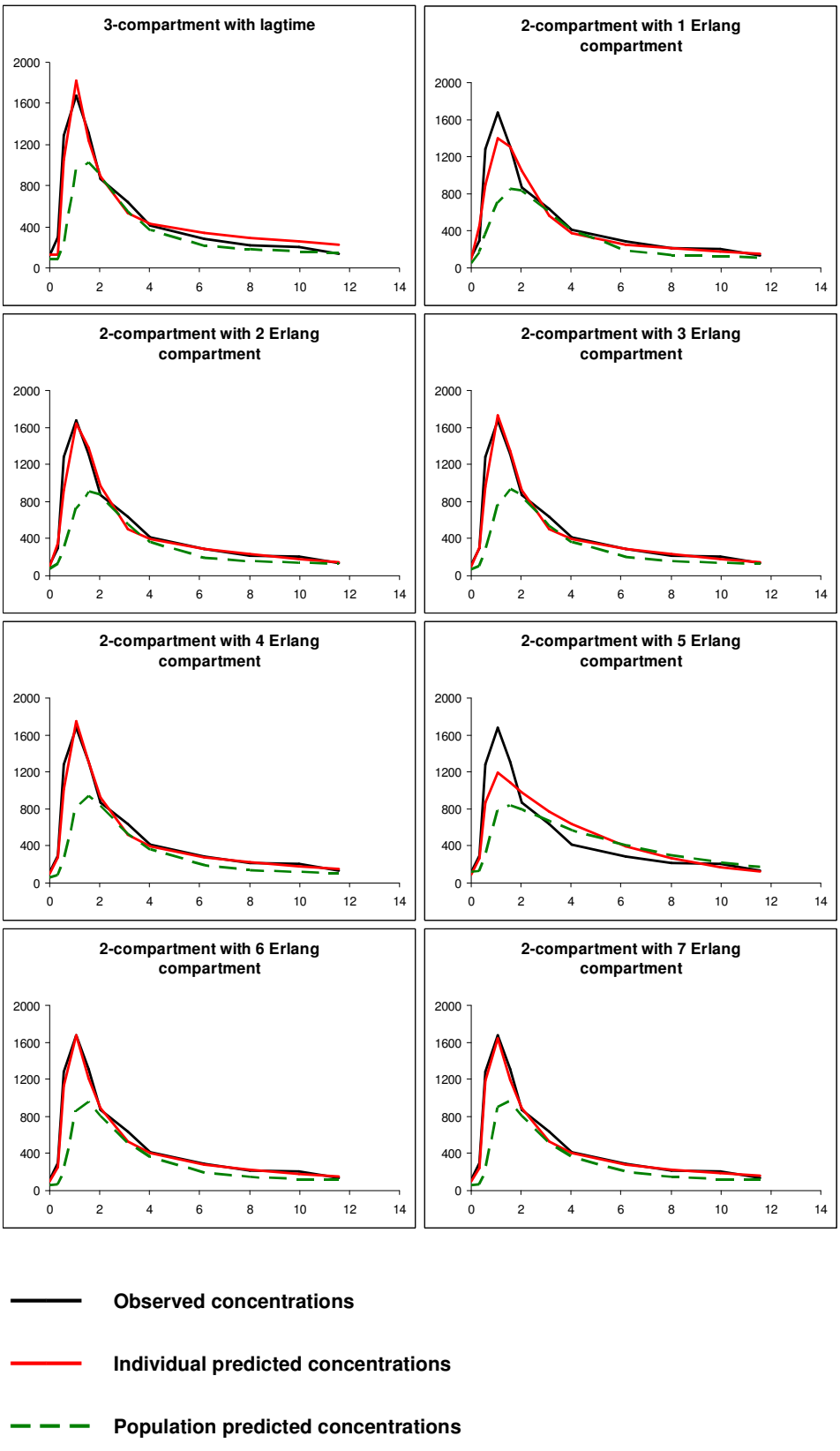


Figure 4. 12-hours PK-profiling of patient no. 70 with the different preliminary models (x = Time (h); y = Plasma concentration (ng/ml))

3.2 ANALYZING FOR COVARIATES

The fixed-effects parameters estimated for the final 2-compartment model were CL/F (θ_1), Q/F (θ_2), V_C/F (V1/F, θ_3), V_P/F (V2/F, θ_4), *k*a (θ_5), and ALAG (θ_6). In the screening process all the covariates were tested individually on each parameter with every possible equation for the covariate in question. All the positive covariates were then double checked for significance in the second screening, before chosen for backwards deletion.

Table 6. The significant covariate CYP 3A5

Covariate	Parameter	Model	OFV	Δ OFV	P
CYP 3A5	**CL**	IF C3A5=1 TVCL=θ(1) / ELSE TVCL=θ(7)	**12398.92**	4.08	< 0.05
	V2	IF C3A5=1 TVV2=θ(4)*θ(7) / ELSE TVV2=θ(4)*θ(8)	**12362.20**	40.80	< 0.01

CYP 3A5 as a covariate did give significant change in OFV. It was however not included in the final model because it did not fit in with the clinical setting of the proposed add-on study.

All the significant covariates that were chosen for further testing with backwards deletion are presented in table 7, and all the covariates that showed significance according to the criteria set for backwards deletion are presented in table 8. These were then included in the final model.

Every covariate tested gave at least one significant change in OFV, expect for type 1 diabetes. Initial screening showed some change, but no significance was seen. A more elaborate coding for diabetes may be needed as some patients had type 2 diabetes before receiving renal transplantation and some patients developed type 2 diabetes after receiving transplantation. The impact of this covariate was not tested for this model.

Table 7. Significant covariates after forward inclusion

Covariate	Parameter	Model	OFV	Δ OFV	P
Age	**V2**	TVV2=θ(4)-θ(7)*(1+AGE/100)	**12398.90**	4.10	< 0.05
BMI	**Q**	TVQ=θ(3)+θ(7)*(BMI-24.86)	**12396.35**	6.65	< 0.01
	V2	TVV2=θ(4)+θ(7)*BMI	**12352.59**	50.41	< 0.01
Creatinine CL	**V1**	TVV1=θ(2)-θ(7)*(CRCL-71.35)	**12362.67**	40.33	< 0.01
	V2	TVV2=θ(4)-θ(7)*(1+CRCL/100)	**12397.35**	5.65	< 0.05
Gender	**CL**	IF GEN=1 TVCL=θ(1)*θ(7) / ELSE TVCL=θ(1)*θ(8)	**12395.43**	7.57	< 0.01
	KA	IF GEN=1 TVKA=θ(5)*θ(7) / ELSE TVKA=θ(5)*θ(8)	**12391.29**	11.71	< 0.01
	V1	IF GEN=1 TVV1=θ(2)*θ(7) / ELSE TVV1=θ(2)*θ(8)	**12395.14**	7.86	< 0.01
	V2	IF GEN=1 TVV2=θ(4)*θ(7) / ELSE TVV2=θ(4)*θ(8)	**12356.30**	46.70	< 0.01
Height	**KA**	TVKA=θ(5)-θ(7)*(HGHT-1.76)	**12392.63**	10.37	< 0.01
	Q	TVQ=θ(3)+θ(7)*(1+HGHT/100)	**12398.81**	4.19	< 0.05
	V1	TVV1=θ(2)+θ(7)*(HGHT-1.76)	**12396.90**	6.10	< 0.05
	V2	TVV2=θ(4)-θ(7)*HGHT	**12397.02**	5.98	< 0.05
LBM	**CL**	TVCL=θ(1)+θ(7)*(LBM-55.74)	**12394.07**	8.93	< 0.01
	V1	TVV1=θ(2)+θ(7)*(LBM-55.74)	**12397.33**	5.67	< 0.05
Steroid dose	**CL**	TVCL=θ(1)+θ(7)*STER	**12292.87**	110.13	< 0.01
	KA	TVKA=θ(5)-θ(7)*(1+STER/100)	**12303.79**	99.21	< 0.01
	V1	TVV1=θ(2)-θ(7)*(1+STER/100)	**12397.74**	5.26	< 0.05
	V2	TVV2=θ(4)+θ(7)*STER	**12398.90**	4.10	< 0.05
Post-transplantation time	**ALAG**	IF TXT<2 TVALAG=θ(6)*θ(7) / ELSE TVALAG=θ(6)*θ(8)	**12210.76**	192.24	< 0.01
	KA	TVKA=θ(5)+θ(7)*TXT	**12147.16**	255.84	< 0.01
	Q	IF TXT<2 TVQ=θ(3)*θ(7) / ELSE TVQ=θ(3)*θ(8)	**12179.73**	223.27	< 0.01
	V1	TVV1=θ(2)-θ(7)*TXT	**12303.54**	99.46	< 0.01
	V2	TVV2=θ(4)-θ(7)*(1+TXT/100)	**12398.98**	4.02	< 0.05
Weight	**V2**	TVV2=θ(4)-θ(7)*WT	**12396.91**	6.09	< 0.05

Table 8. Significant covariates after backwards deletion

Covariate	Parameter	Model	OFV	Δ OFV	P
BMI	**V2**	TVV2=θ(4)+θ(29)*BMI	**11985.76**	12.84	< 0.01
Creatinine CL	**V1**	TVV1=θ(2)-θ(21)*(CRCL-71.35)	**12078.80**	105.88	< 0.01
Gender	**CL**	IF GEN=1 TVCL=θ(1)*θ(7) / ELSE TVCL=θ(1)*θ(8)	**11989.95**	17.03	< 0.01
	KA	IF GEN=1 TVKA=θ(5)*θ(15) / ELSE TVKA=θ(5)*θ(16)	**12051.13**	78.21	< 0.01
	V1	IF GEN=1 TVV1=θ(2)*θ(9) / ELSE TVV1=θ(2)*θ(10)	**12167.10**	194.18	< 0.01
	V2	IF GEN=1 TVV2=θ(4)*θ(13) / ELSE TVV2=θ(4)*θ(14)	**11992.99**	20.07	< 0.01
Height	**KA**	TVKA=θ(5)-θ(30)*(HGHT-1.76)	**11980.58**	7.66	< 0.05
	Q	TVQ=θ(3)+θ(26)*(1+HGHT/100)	**11986.33**	13.41	< 0.01
	V1	TVV1=θ(2)+θ(22)*(HGHT-1.76)	**12096.86**	123.94	< 0.01
LBM	**CL**	TVCL=θ(1)+θ(20)*(LBM-55.74)	**11980.77**	7.85	< 0.05
	V1	TVV1=θ(2)+θ(25)*(LBM-55.74)	**11988.55**	15.63	< 0.01
Steroid dose	**CL**	TVCL=θ(1)+θ(19)*STER	**12086.09**	113.17	< 0.01
	KA	TVKA=θ(5)-θ(31)*(1+STER/100)	**12076.88**	103.96	< 0.01
	V1	TVV1=θ(2)-θ(24)*(1+STER/100)	**12042.40**	69.48	< 0.01
	V2	TVV2=θ(4)+θ(28)*STER	**12005.42**	32.50	< 0.01
Post-transplantation time	**ALAG**	IF TXT<2 TVALAG=θ(6)*θ(17) / ELSE TVALAG=θ(6)*θ(18)	**11980.60**	7.68	< 0.05
	KA	TVKA=θ(5)+θ(32)*TXT	**12177.61**	204.69	< 0.01
	Q	IF TXT<2 TVQ=θ(3)*θ(11) / ELSE TVQ=θ(3)*θ(12)	**12094.28**	121.36	< 0.01
	V1	TVV1=θ(2)-θ(23)*TXT	**11994.78**	21.86	< 0.01
Weight	**V2**	TVV2=θ(4)-θ(27)*WT	**12090.99**	118.07	< 0.01

OFV in the start model was 12403.00 and dropped to 11972.92 when all the significant covariates were added. After the backwards deletion the final model had an OFV of 12088.50, which is highly significantly better than the start model.

3.2.1 Covariate analysis based on visual and graphical representation

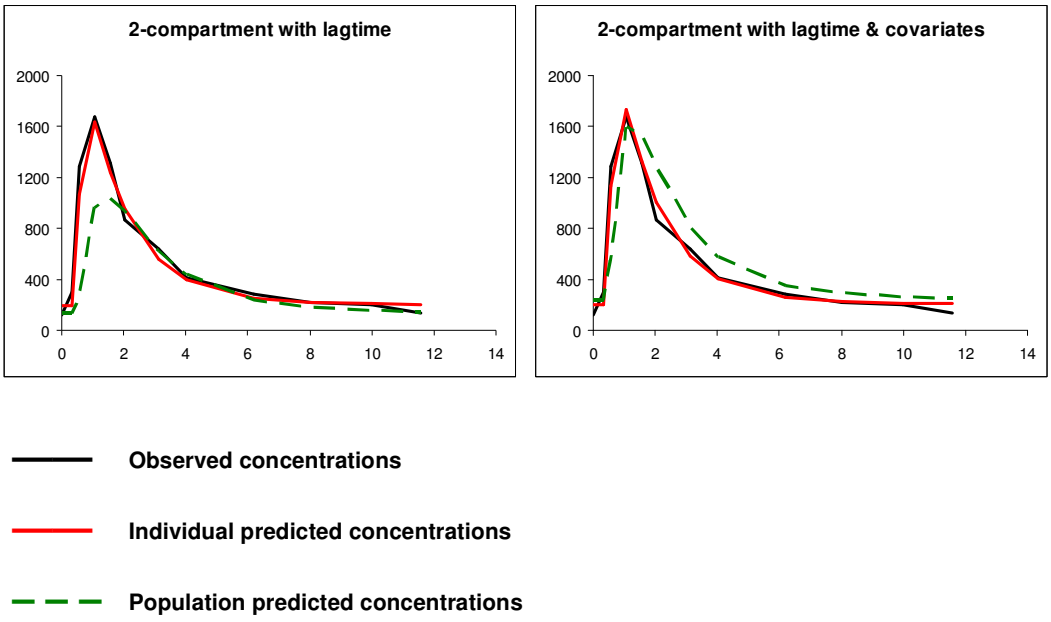


Figure 5. Comparison of start model to the final model in patient no. 70s full 12-hours PK-profiling (x = Time (h); y = Plasma concentration (ng/ml))

Patient no. 70 showed the greatest improvement in fit after inclusion of covariates. When comparing the start model to the full covariate model it is clear that the population predicted concentrations are corrected according to the covariates. The peak concentrations are more accurate and no longer underestimated.

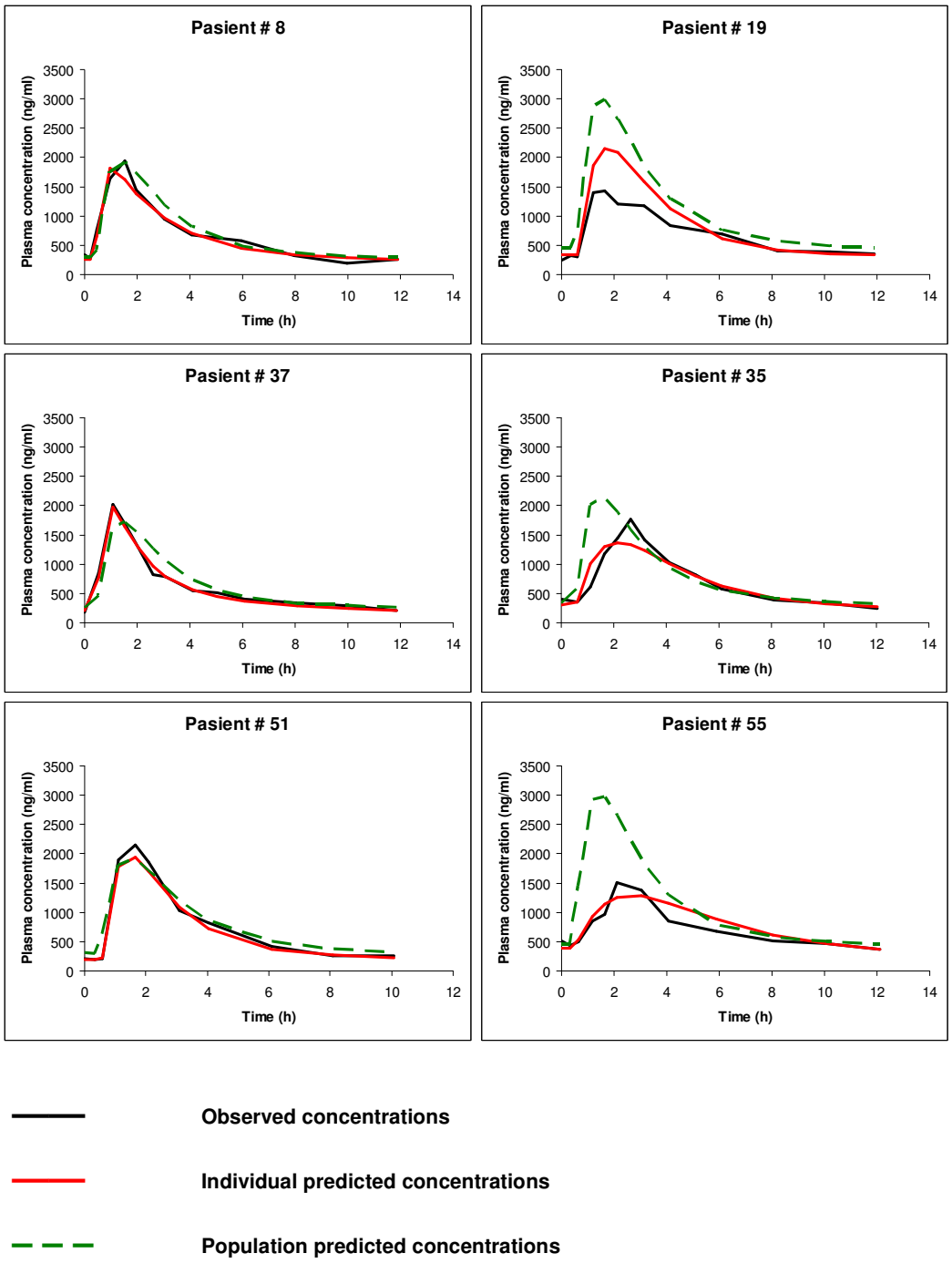


Figure 6. Plots showing the best (left) and worst (right) fits of the start model for PK-profiling patients

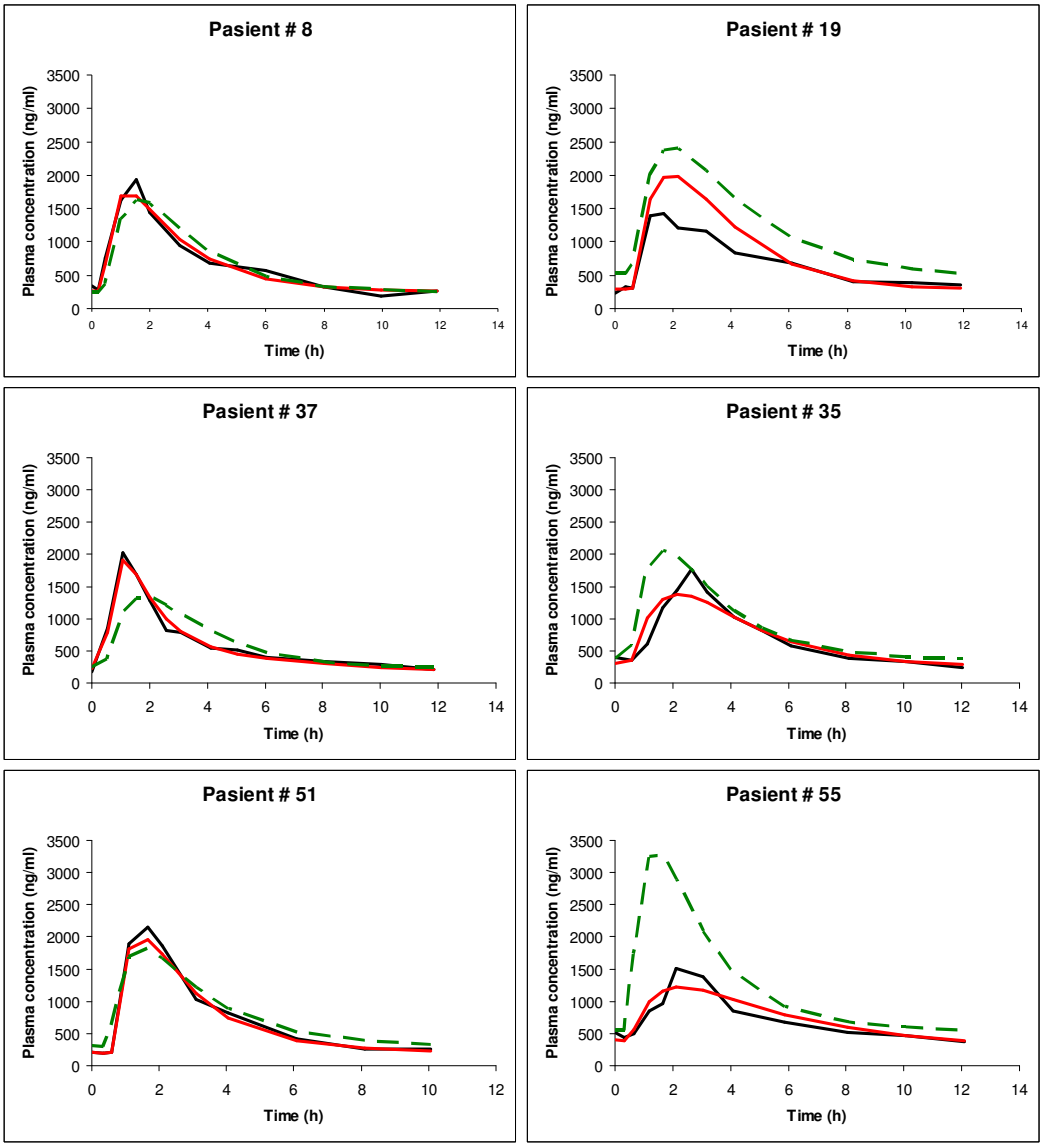


Figure 7. Plots showing the best (left) and worst (right) fits of the start model after inclusion of covariates into the final model

The difference in the plots of figure 6 and 7 are not apparent and not evident visually. Though the effect of covariate addition is evident in Δ OFV presented in table 4 and the goodness of fit (GOF) plots (Figure 8).

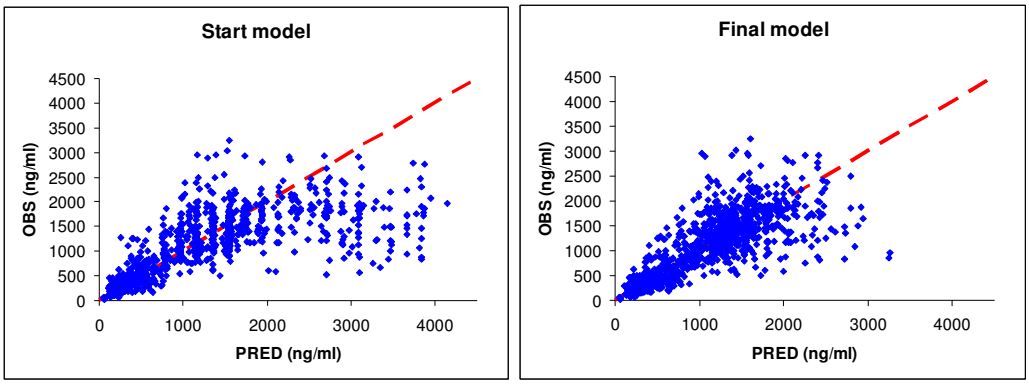


Figure 8. Goodness of fit plot of observed vs. population predicted concentrations in start and final model

The start model without covariates has a tendency to over-predict low CsA concentrations which reduces the fit significantly. Addition of covariates to the model greatly improves the fit.

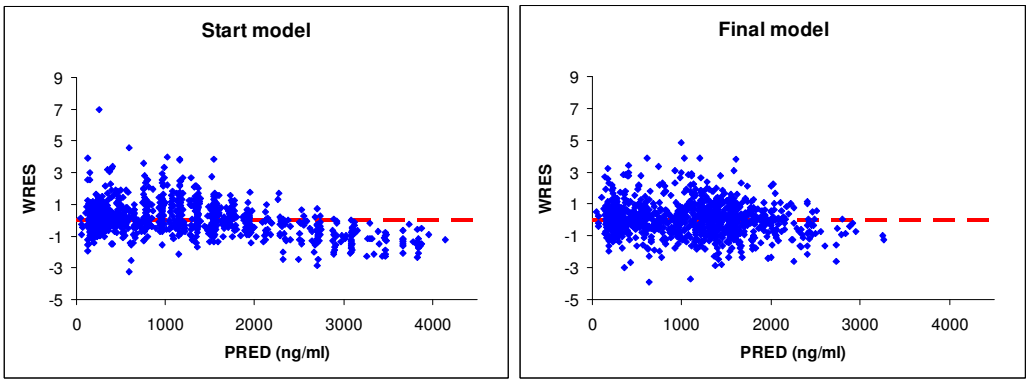


Figure 9. Plot of weighted residuals vs. population predicted concentrations in start and final model

Diagnostic plots of the weighted residuals shows that minimum / maximum WRES range drop from -3.2339 / 7.0121 in the start model to -3.8828 / 4.8198 in the final model with covariates.

3.3 THE FINAL MODEL FOR CYCLOSPORIN A

The model that best described the data, based on the above mentioned criteria, was a 2-compartment model with first order absorption, first order elimination and an absorption lagtime. A combined additive and proportional model was used to describe the distribution of random residual error, and an exponential error model described the inter-individual variability. The first-order conditional estimation method gave the lowest OFV.

```
$PROBLEM ORAL ADMIN., 2 COMP., 1. ORDER ABS., WITH ABS. LAGTIME; Created by Truc Van Le

$DATA Input.FINAL.FULL.MODEL.txt

$INPUT ID AMT RATE DAT1=DROP TIME C=DV WT UREA=DROP SCR=DROP CRCL MDV SS II CMT FLAG AGE GEN HGHT
TXT STER BMI C3A5=DROP LBM

$SUBROUTINE ADVAN4

$PK

   IF (GEN.EQ.1) THEN
   TVCL=THETA(1)*THETA(7)+THETA(19)*STER+THETA(20)*(LBM-55.74)
   ELSE
   TVCL=THETA(1)*THETA(8)+THETA(19)*STER+THETA(20)*(LBM-55.74)
   ENDIF

   IF (GEN.EQ.1) THEN
   TVV1=THETA(2)*THETA(9)-THETA(21)*(CRCL-71.35)+THETA(22)*(HGHT-1.76)-THETA(23)*TXT-
THETA(24)*(1+STER/100)+THETA(25)*(LBM-55.74)
   ELSE
   TVV1=THETA(2)*THETA(10)-THETA(21)*(CRCL-71.35)+THETA(22)*(HGHT-1.76)-THETA(23)*TXT-
THETA(24)*(1+STER/100)+THETA(25)*(LBM-55.74)
   ENDIF

   IF (TXT.LT.2) THEN
   TVQ=THETA(3)*THETA(11)+THETA(26)*(1+HGHT/100)
   ELSE
   TVQ=THETA(3)*THETA(12)+THETA(26)*(1+HGHT/100)
   ENDIF

   IF (GEN.EQ.1) THEN
   TVV2=THETA(4)*THETA(13)-THETA(27)*WT+THETA(28)*STER+THETA(29)*BMI
   ELSE
   TVV2=THETA(4)*THETA(14)-THETA(27)*WT+THETA(28)*STER+THETA(29)*BMI
   ENDIF

   IF (GEN.EQ.1) THEN
   TVKA=THETA(5)*THETA(15)-THETA(30)*(HGHT-1.76)-THETA(31)*(1+STER/100)+THETA(32)*TXT
   ELSE
   TVKA=THETA(5)*THETA(16)-THETA(30)*(HGHT-1.76)-THETA(31)*(1+STER/100)+THETA(32)*TXT
   ENDIF

   IF (TXT.LT.2) THEN
   TVALAG=THETA(6)*THETA(17)
   ELSE
   TVALAG=THETA(6)*THETA(18)
   ENDIF

   CL=TVCL*EXP(ETA(1))                ;Clearance (CL/F) L/hr
   V1=TVV1*EXP(ETA(2))                ;Central volume (V1/F), L
   Q=TVQ*EXP(ETA(3))                  ;Intercompartmental clearance (Q/F)
   V2=TVV2*EXP(ETA(4))                ;Peripheral volume (V2/F), L
   KA=TVKA*EXP(ETA(5))                ;Absorption rate constant, 1/hr
   ALAG1=TVALAG*EXP(ETA(6))           ;Absorption lag time, hr

   S2=V1
```

```
   K=CL/V1
   K23=Q/V1
   K32=Q/V2

$ERROR
   IPRED=F
   Y=F+F*ERR(1)+ERR(2)

$THETA (23,26.1,40)      ;THETA(1) is POPCL/F
$THETA (75,77.9,95)      ;THETA(2) is POPV1/F
$THETA (15,20.4,30)      ;THETA(3) is POPQ/F
$THETA (320,342,400)     ;THETA(4) is POPV2/F
$THETA (.5,1.86,3)       ;THETA(5) is POPKA
$THETA (0.2,0.444,0.7)   ;THETA(6) is POPLAGTIME
$THETA (0.01,1)          ;THETA(7) is Effect of GEN on CL, male
$THETA (0.01,1)          ;THETA(8) is Effect of GEN on CL, female
$THETA (0.01,1)          ;THETA(9) is Effect of GEN on V1, male
$THETA (0.01,1)          ;THETA(10) is Effect of GEN on V1, female
$THETA (0.01,1)          ;THETA(11) is Effect of TXT on Q, < 2 weeks
$THETA (0.01,1)          ;THETA(12) is Effect of TXT on Q, >= 2 weeks
$THETA (0.01,1)          ;THETA(13) is Effect of GEN on V2, male
$THETA (0.01,1)          ;THETA(14) is Effect of GEN on V2, female
$THETA (0.01,1)          ;THETA(15) is Effect of GEN on KA, male
$THETA (0.01,1)          ;THETA(16) is Effect of GEN on KA, female
$THETA (0.01,1)          ;THETA(17) is Effect of TXT on ALAG, < 2 weeks
$THETA (0.01,1)          ;THETA(18) is Effect of TXT on ALAG, >= 2 weeks
$THETA (0.01,0.5)        ;THETA(19) is Effect of STER on CL
$THETA (0.01,0.5)        ;THETA(20) is Effect of LBM on CL
$THETA (0.01,0.5)        ;THETA(21) is Effect of CRCL on V1
$THETA (0.01,0.5)        ;THETA(22) is Effect of HGHT on V1
$THETA (0.01,0.5)        ;THETA(23) is Effect of TXT on V1
$THETA (0.01,0.5)        ;THETA(24) is Effect of STER on V1
$THETA (0.01,0.5)        ;THETA(25) is Effect of LBM on V1
$THETA (0.01,0.5)        ;THETA(26) is Effect of HGHT on Q
$THETA (0.01,0.5)        ;THETA(27) is Effect of WT on V2
$THETA (0.01,0.5)        ;THETA(28) is Effect of STER on V2
$THETA (0.01,0.5)        ;THETA(29) is Effect of BMI on V2
$THETA (0.01,0.5)        ;THETA(30) is Effect of HGHT on KA
$THETA (0.01,0.5)        ;THETA(31) is Effect of STER on KA
$THETA (0.01,0.1)        ;THETA(32) is Effect of TXT on KA

$OMEGA
0.07     ;BSVCL/F
0.2      ;BSVV1/F
0.1      ;BSVQ/F
2.35     ;BSVV2/F
0.315    ;BSVKA
0.0085   ;BSVALAG1

$SIGMA 0.0662   ;ERRCV, Proportional error (%)
$SIGMA 24.4     ;ERRSD, Additive error (ug/ml)

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$COVARIANCE

$TABLE ID TIME DV IPRED MDV
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID CL V1 Q V2 KA ALAG1 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 WT CRCL FLAG AGE GEN HGHT TXT STER
BMI LBM
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

Figure 10. Control file for the final model with all significant covariates

Figure 10 shows the full control file with all the significant covariates. Also included are the starting estimates for THETA, OMEGA and SIGMA values, used to estimate fixed effects, inter-individual variability and residual variability, respectively.

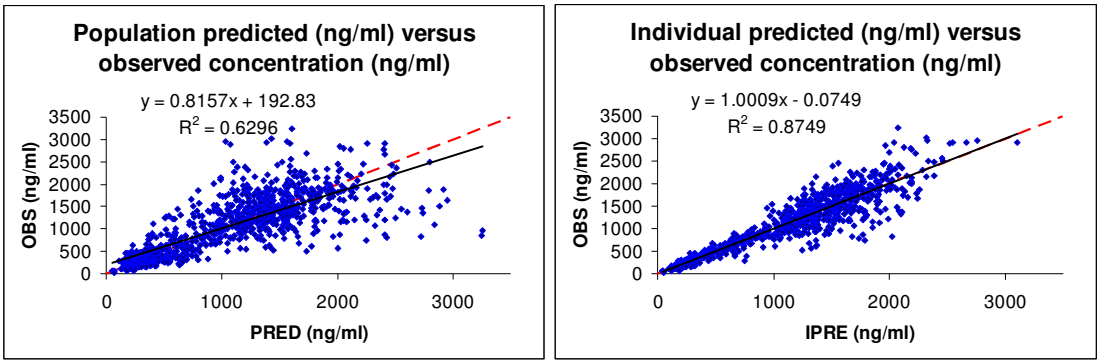


Figure 11. Goodness of fit scatter plot of population predicted (PRED) and individual predicted (IPRE) vs. observed concentrations (OBS) of the final model

GOF plots in figure 11 shows a good fit for the data. No significant deviation is seen in either population predicted or individual predicted plots.

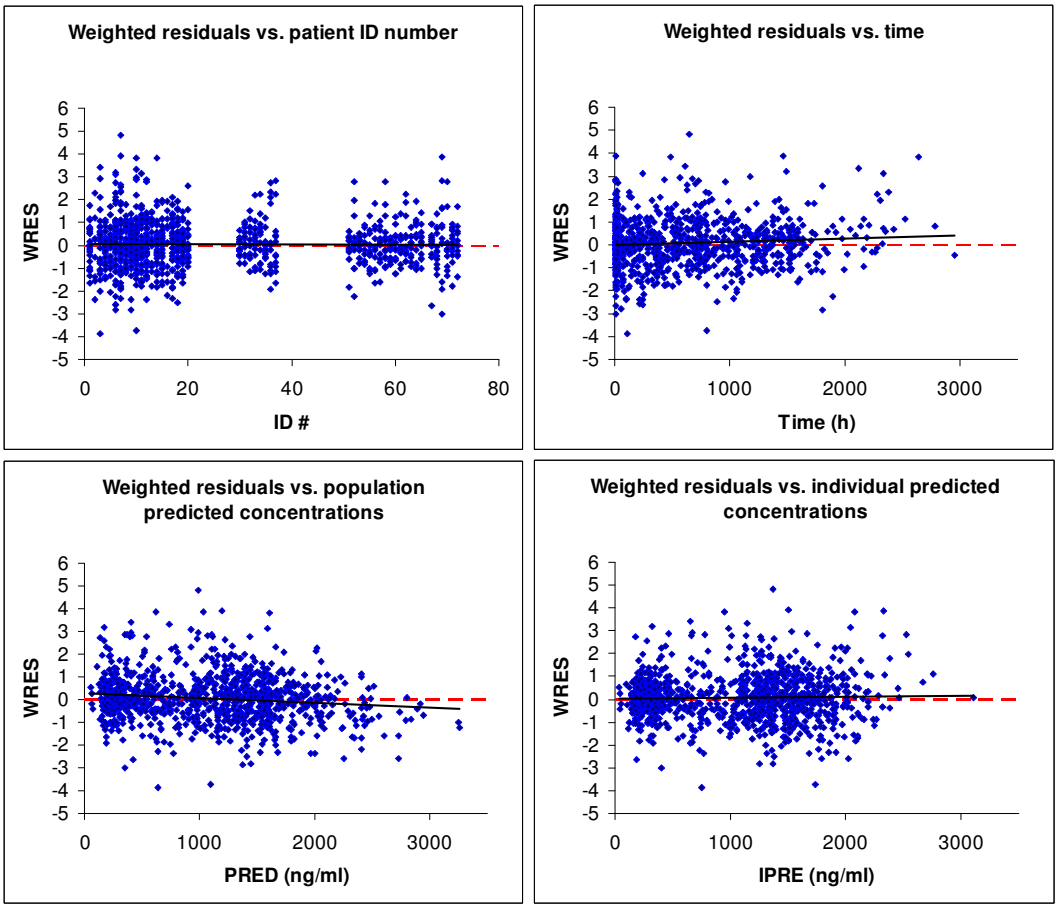


Figure 12. Scatter plot of weighted residuals of the final model (ID# 1-20 = SUPER-CsA study, ID# 30-37 = MIMPARA study, ID# 51-65 & 67-72 = Age effect study)

3.4 VALIDATING THE MODEL

3.4.1 Internal validation

The parameter estimates obtained from the data-splitting are presented in figure 13. The majority of the estimated values are within ± 1 standard deviation. However, CL/F and ALAG does have one value each that is outside of ± 2 standard deviation.

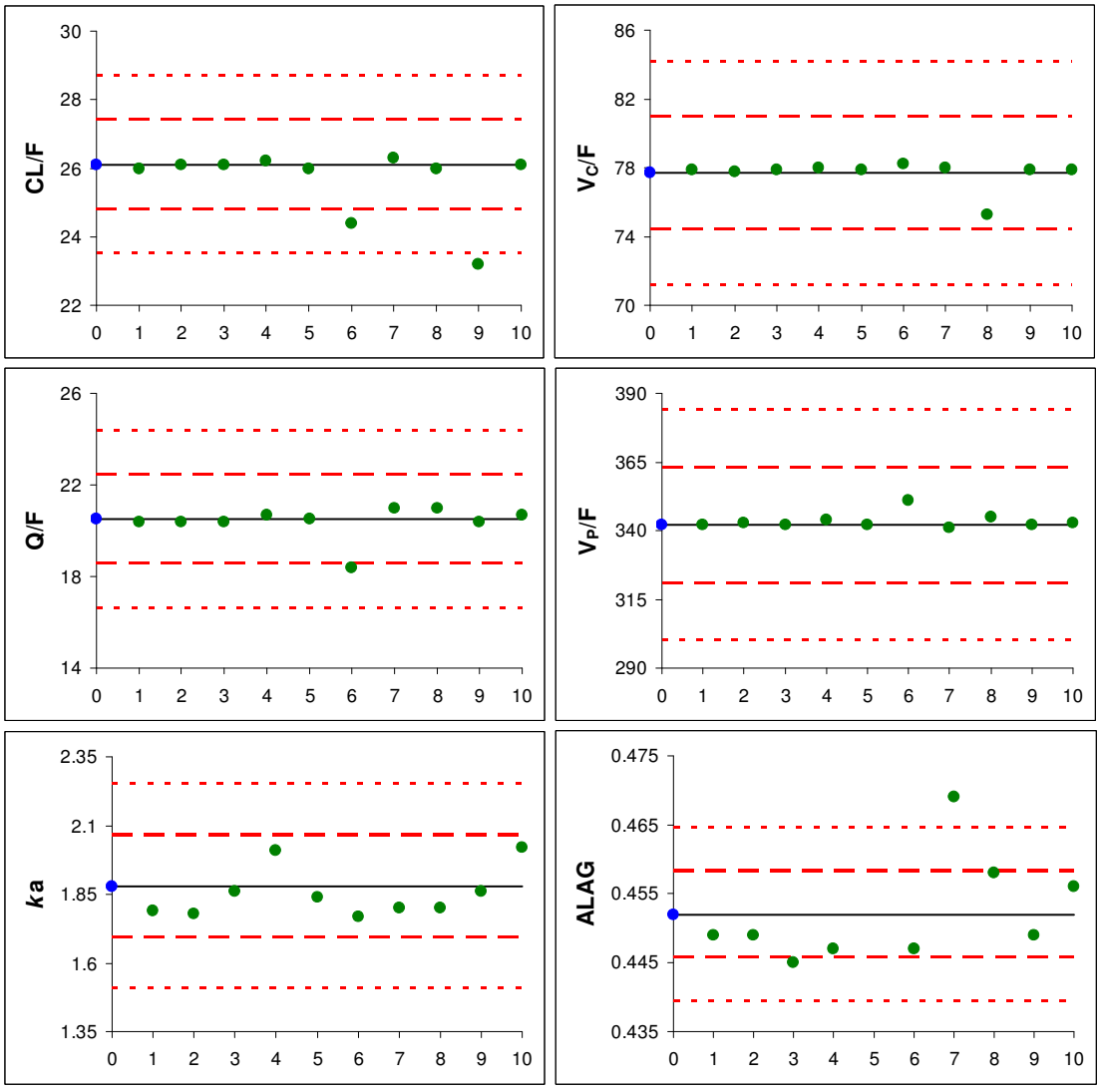


Figure 13. Parameter estimates for the full data set (●) and the ten subsets containing ~90 % of the population data (●) with ± 1 SD (– –) and ± 2 SD (- - -)

3.4.1.1 Examining the predictive performance of the population model

Table 9. The prediction errors for predicted concentrations using all / none / only the first / the first and second available observed concentrations

		ALL	NONE	1st	1st & 2nd
ME (ng/ml)	**Mean**	-7.25	-42.71	-69.30	-66.66
	SD	28.27	127.88	139.99	135.95
	95 % CI min.	-24.77	-121.97	-156.07	-150.93
	95 % CI max.	10.27	36.55	17.47	17.60
MSE (ng/ml)^2	**Mean**	64510.18	183866.57	188016.22	180250.51
	SD	17211.28	44022.25	61443.44	48174.34
	95 % CI min.	53842.52	156581.29	149933.18	150391.74
	95 % CI max.	75177.84	211151.84	226099.26	210109.28
RMSE (ng/ml)	**Mean**	164.49	283.80	287.28	281.61
	SD	20.80	34.42	49.52	38.99
	95 % CI min.	151.60	262.47	256.59	257.44
	95 % CI max.	177.38	305.14	317.97	305.77
AVERAGE SPE_{ALL}	**Mean**	0.01	0.09	0.15	0.14
	SD	0.05	0.24	0.25	0.23
	95 % CI min.	-0.02	-0.05	-0.01	0.00
	95 % CI max.	0.04	0.24	0.30	0.28
SD SPE_{ALL}	**Mean**	0.41	0.63	0.65	0.63

Abbreviations; ME = mean prediction error, MSE = mean squared prediction error, RMSE = root mean squared error, SPE = standardized prediction error

ME is used as a measure of bias and from table 9 it can be seen that the individual predicted concentrations have a slight tendency to be underestimated.

The precision of the model is assessed by MSE and RMSE. Clearly the data set with all the available observed concentrations show the greatest precision. The data sets with none, only the first, or the first and second observation does not show any large degree of difference between them, but have significantly less predictive performance than the data set with all observations.

Predictive performance was also tested with standardized prediction error. The estimated average values were all very close to zero, which is a good indicator for good fit. The 95 % CI should also include zero, which all the data sets did. Not surprisingly the full data set performed the best. Standard deviation of SPE should be as close to 1 as possible to give the

best predictive performance. The model falls short in this test, and has lower values than desired [18].

Table 10. Objective function values of full input model with fixed parameter estimates from subgroups A-J

OFV value					
	Final model		12088.50	**Mean**	**Minimum**
	Subgroups				
	Fixed parameter values from included subset (~90 %) on full input file	**A**	12116.57		
		B	12116.74	12108.65	12001.52
		C	12254.61	**Median**	**Maximum**
		D	12020.62		
		E	12227.53		
		F	12021.83	12098.04	12254.61
		G	12079.51	**Standard deviation**	**Standard error of mean**
		H	12001.52		
		I	12193.07		
		J	12054.54	90.21	28.53

A 95 % CI is obtained when OFV does not differ more than ≥ 3.84. In the developed model the OFV obtained from the final model varied greatly, when run with fixed parameter estimates from the 10 subgroups. Values ranged from 12001.52 to 12254.61, which indicate a lack of robustness.

3.4.2 Confidence interval

Table 11. Results from the Jackknife

PK parameters	Mean	SD	95 % CI min.	95 % CI max.
Clearance	25.54	1.30	25.18	25.91
Central volume	78.69	3.25	77.78	79.60
Intercompartmental clearance	20.53	1.95	19.99	21.08
Peripheral volume	351.5	20.99	345.7	357.4
Absorption rate constant	1.872	0.190	1.820	1.924
Absorption lagtime	0.4495	0.0063	0.4477	0.4512

Upon reviewing the individual Jackknife estimates (Appendix 7.7), it showed that the patients did not exhibit any significant influence on the parameters.

3.4.3 External validation

Table 12. The prediction errors for predicted concentrations in the external group

		ALL	NONE	1st conc.	1st week	1st & 2nd weeks
ME (ng/ml)	**Mean**	13.19	18.93	-2.10	8.20	-4.45
	SD	28.33	136.76	234.26	171.74	117.23
	95 % CI min.	-4.37	-65.83	-147.30	-98.25	-77.11
	95 % CI max.	30.75	103.70	143.09	114.64	68.21
MSE $(ng/ml)^2$	**Mean**	68162.36	94782.70	124617.96	100785.97	82416.73
	SD	42435.27	47882.74	70716.91	49887.68	48767.54
	95 % CI min.	41860.71	65104.67	80787.17	69865.27	52190.30
	95 % CI max.	94464.01	124460.74	168448.76	131706.68	112643.16
RMSE (ng/ml)	**Mean**	188.79	242.37	280.85	249.56	223.24
	SD	56.98	69.30	78.59	65.95	70.43
	95 % CI min.	153.47	199.42	232.14	208.68	179.59
	95 % CI max.	224.11	285.32	329.55	290.44	266.89
AVERAGE SPE_{ALL}	**Mean**	-0.04	-0.04	0.01	-0.06	-0.06
	SD	0.08	0.48	0.76	0.48	0.44
	95 % CI min.	-0.09	-0.34	-0.46	-0.36	-0.33
	95 % CI max.	0.01	0.26	0.48	0.23	0.22
SD SPE_{ALL}	**Mean**	0.78	0.89	0.88	0.81	0.82

Abbreviations; ME = mean prediction error, MSE = mean squared prediction error, RMSE = root mean squared error, SPE = standardized prediction error

As expected, by including more concentration data points into the data set the prediction errors decrease. The amount of data points per week were between 3-4 observed concentrations. This was much more than the amount of data included for the internal validation, and thus the last column in table 9 and the two last columns in table 12 should not be compared directly.

The 95 % CI for the standardized prediction error average included zero and the standard deviation was close to 1, indicating a good fit of the data.

4 DISCUSSION

4.1 MODEL BUILDING

Creating the input file necessary for NONMEM was a challenging task. 23 columns and 5973 rows were filled with patient data. The input file was created and then double checked by another person to ensure validity of the file.

A literature search in ISI Web of Knowledge^{SM} and PubMed gave no hits on PK population models using the same type of data set as the one used here. Previous studies have used one full 12-hours PK-profiling [26], three full12-hours PK-profiling performed at 3 weeks, 6 months and 1 year post-transplantation [28], one full 24-hours PK-profiling [29], or limited or rich sampling strategy for AUC-estimation and PK-profiling [27, 41]. The data set used for this model differs from other previous studies by incorporating a large amount of CsA C_0 and C_2 concentration data before and after PK-profiling for the SUPER-CsA patients [43].

Creating the control file with the desired compartment model proved to be difficult. Guides provided by http://www.accp1.org/pharmacometrics/index.html [61] and control files provided by Live Storehagen [50] were used as a basis to build the necessary steps in the control files. More elaborate modifications of the control files were done using the user guide provided with the NONMEM application [7].

As mentioned earlier, many different PK population models have been developed for modelling of CsA. The varying type and amount of data used for the input file may result in different models which best describes the data set in question. A study performed by Wu et al. used a 1-compartment model to describe the CsA data. It was not mentioned if any other models were tested for their data set. This seemed unusual as most other studies use a more complex model than that. A highly lipophilic drug such as CsA would most likely need a peripheral compartment, therefore the model screening process was most focused on 2- and 3-compartment models. Highly inconsistent reporting of model specifications meant that the screening process had to be widened to include many different absorption methods.

4.1.1 1. order input, 1-compartment with and without lagtime

ADVAN2 and TRANS2 subroutine was used to code the control file for the 1-compartment models. The models did not show a good fit for the data. With both individual and population predicted concentrations being strongly underestimated around peak concentrations and overestimated beyond C_2. Inclusion of lagtime did improve this to some extent. OFV for the 1-compartment model without lagtime was 13035.80 and dropped to 12807.03 when lagtime was included. The significance of lagtime meant that it should be tested in all later models. The run times for these two models were very fast, with an average of less than five minutes [19].

4.1.2 1. order input, 2-compartment without lagtime

The subroutine for 2-compartment model was coded with ADVAN4 and TRANS4. A slightly lower OFV of 12805.39 was not statistically significant, but the better fit of the elimination profile justified further testing of this model [19].

4.1.3 1. order input, 2-compartment with lagtime

Subroutine coding for this model was similar to the regular 2-compartment with just the lagtime function added. This model showed a good fit for the data, but peak concentrations was not reached by predicted concentrations and needed addressing. The model used the FOCE estimation method and eventually reached lowest OFV at 12403.00. When tested with the FO estimation method the resulting performance was significantly less, with a rise in OFV to 12553.47. Later models were therefore only coded with FOCE. The model did have a problem with instability between runs in the beginning. By making the interval for the starting estimates of the parameters smaller, the model did performed better without hitting the limits.

4.1.4 0. order input, 2-compartment with lagtime

ADVAN3 was used to code the subroutine of the 2-compartment model with zero order input. The model showed highly variable estimates of the parameter and a high OFV of 12954.06. The serious lack of robustness of this model meant that it was discarded in favor of other models. The run time for the 2-compartment models up until this point were good and most runs finished between 10-20 minutes [19].

4.1.5 1. order input, 3-compartment with and without lagtime

The 3-compartment models were coded with subroutine ADVAN12. The model without lagtime did not perform well with an OFV of 12776.7 and a runtime averaging at 50 minutes. Peak concentrations were not well predicted. Inclusion of lagtime showed improvement much like in the other models. OFV dropped to 12457.45, but run time increased with approximately 20 minutes. Though initial model testing showed that the 3-compartment model gave a low OFV value, it was not chosen for further investigation mainly due to its instability. Small changes in the start values for parameter estimates would throw the model off course, and yield noticeable change in model performance and parameter estimation [19].

4.1.6 2-compartment with Erlang distribution

To incorporate the Erlang distribution into the 2-compartment control file, a more complex subroutine was used. ADVAN5 SS5 was used to make the user defined Erlang absorption profile. Describing the data using the Erlang distribution did produce a good fit for the delayed and flat absorption profile of CsA, but severely overcomplicated the model. Run time increased from an average of 30 minutes for a regular 2-compartment model with lagtime to between 4-24 hours for the acceptable models with Erlang distribution. Compared to the final model, the Erlang model had a higher OFV value. The number of Erlang compartments was tested according to the criteria mentioned in paragraph 2.2. The resulting OFVs showed that improvement could be seen from 1 to 4 Erlang distribution compartments. Adding the fifth Erlang compartment, while retaining the same start values for the parameter estimates, gave a significant rise in OFV. When adding the sixth Erlang

compartment a drop in OFV was seen. The downside was that runtime from this point on exceeded 72 hours. To be useful in the clinical add-on study, runtime would have to be approximately 24 hours, as the model would be set to run over night, and results would be needed for the next morning. Adding more compartments after this point did not show any improvement in fit, and gave an increase of OFV [19, 27, 50].

Storehagen [50] used the PK-profiling of the SUPER-CsA study [43] and the MIMPARA study [45] and found the Erlang distribution with 6 Erlang compartments to describe the data the best. With only these data the model also performed much faster, with the run time (29 minutes and 15 seconds) well below the criteria set for the model developed in this thesis. The difference is most likely due to the amount of data used by Storehagen [50], 210 observed concentrations, compared to the amount of data used for the model in this thesis, 1027 observed concentrations. The PK-profiling is done no closer than one month post-transplantation, so that the patients have had time to stabilize their physiology. The data set used in this thesis was from 0 weeks and up to 17 weeks post-transplantation. There are large time dependent variations that are possibly overlooked when using only PK-profiling. The large amount of data used in this thesis may possibly require simpler models to match the criteria set, while less data can employ more elaborate models without defying the same criteria.

4.2 ANALYZING FOR COVARIATES

Reviewing the graphical representation of post hoc etas vs. covariates (Appendix 7.3) shows that many covariates influences the PK parameters of the model. However, when screening for covariates estimation show that most of the covariates are in fact not statistical significant. Many covariates did show significance during forward inclusion, but most eventually did not prove to be significant when run through backwards deletion.

As mentioned earlier, Storehagen [50] used some of the data from the same studies as used in this thesis. The covariate screening for that study included weight (kg), creatinine clearance (ml/min), age (years), height (cm), gender, post-transplantation period (weeks) and steroid dose at the pharmacokinetic day (mg). The graphical analysis then showed that weight, age and creatinine clearance tended to correlate with some of the PK parameters, and

therefore needed further investigation. Though ultimately, the only significant covariate was age on CL/F [50]. The large amount of significant covariates for the developed model in this thesis is therefore surprising as also many other studies have only a few or no significant covariates, but this may be due to the large amount of data over a relatively long time period available in the present study [18, 26-29, 42].

All linear models gave significant results on some combinations of covariate and parameter. Screening with the proportional models did not give any significant results.

In the preliminary models it was evident that approximately the first 16 days after transplantation showed signs of highly variable concentration predictions. This could only be seen in the patients from the SUPER-CsA study, which provided more data than just PK-profiling. It was decided to test more rigorously for any relationship between predicted concentrations and covariates influencing this time period. The goodness of fit plots in figure 8 of the start model shows difficulty in modelling this first time period after transplantation. Concentrations are highly over predicted as can be seen in the quality of fit plots (Observed/Predicted over time) of the start model (Appendix 7.4). Many low plasma concentrations during this time are predicted wrongly by a factor of approximately two. The early periods after transplantation are plagued with the body going through a lot of stress. The operation procedure for renal transplantation includes going through the stomach which will most definitely impact the absorption and bioavailability of CsA. Altered gastrointestinal motility is to be expected. A factor that might also influence this time period is the switch from i.v. Solu-Medrol® during transplantation to oral prednisolone after transplantation. These influences are reflected by the high variability in observed concentration. Though it should not be ruled out that assay may also play a role. By incorporating time dependent covariates such as post-transplantation time and steroid dose, much of this variability is accounted for. [62] GOF plots of the final model (Figure 8 and Figure 11) clearly show an improvement in fit with a more even distribution. Steroid dose (mg), in the form of prednisolone, proved to be significant on CL/F, *k*a, V1/F and V2/F, while post-transplantation time (weeks) was significant when applied to ALAG, *k*a, V1/F and V2/F. Some of this was expected as Saint-Marcoux et al. [26], Wu et al. [18] and Rosenbaum et al. [35] have already incorporated post-transplantation time in PK population models for CsA.

Covariates such as BMI, gender, height, post-transplantation time and steroid dose gave many significant changes in OFV of the start model. They were tested with many different equations, and gave overlapping answers. Only a few were selected for backwards deletion. The choices were based on which covariate that gave the largest drop in OFV, and the clinical significance of the covariate was also weighted.

The effect of weight, BMI and LBM on volume parameters were significant, not surprisingly. Patients commonly gain weight up to 10 % after transplantation. This increase can be attributed to decreased physical activity coupled with inappropriate food intake, also possibly due to administration of high doses of steroids, and the move from a catabolic end stage renal disease (ESRD) phase to a more anabolic post-transplantation phase. [18]

Gender did produce significant changes in OFV when added to the model. Significance was shown for CL/F, *k*a, V_C/F and V_P/F. This was surprising as most other studies did not find this covariate to improve models. [18, 34] When reviewing the demographics for the two genders, it shows that there is not much difference in mean values for age, height or BMI. Females do however have a significantly lower weight of ~15 kg and this correlates with the LBM being ~10 kg lower. This weight difference could be the source of gender as a significant covariate.

Storehagen [50] and Wu et al. [18] have previously shown age as a significant covariate for CL/F. Physiological changes related to increasing age include loss of liver mass and reduced blood flow to the liver, which influences the metabolism and clearance of CsA [44, 50]. Age was therefore expected to be a significant covariate, but this could not be seen in this population. Preliminary screening indicated some significance, but age as a continuous covariate did not prove to be significant after backwards deletion.

As mentioned earlier, CYP 3A5 genotype did give a significant change in OFV during forward inclusion. Even though it should be tested further for possible inclusion in the final model with all the significant covariate, it was left out because of the analysis required for genotype identification is not clinically available yet. This rendered it not efficient enough to be used in the clinical setting of the proposed add-on study for external validation. This being said, it may prove to be useful for later studies. It was also mentioned that CYP enzymes work in concert with P-gp

transporters. Though the effect of P-gp mutations might have influenced the CsA data, it was not tested for this model.

Diabetes did not prove to be a significant covariate, but this may be due to the fact that it was not detailed specifically. The coding for diabetes included only non-diabetic = “FLAG=1” or type 1 diabetes = “FLAG=2”. As some patients also had type 2 diabetes before undergoing renal transplantation and some patients developed type 2 diabetes after the transplantation, a more specific coding for diabetes might have proven useful for the model. This was however not tested for this model.

Overall the peak concentrations for the PK-profiling patients were estimated more accurately after inclusion of covariates into the model. Individual predications were not markedly affected, while population predictions show signs of improvement. The most notable effect of covariate inclusion is the greatly improved fit in the first time period after transplantation. Although many sources of variability was identified and quantified, there is still some variability that is unaccounted for in this present model. Differentiation of oral prednisolone and i.v. Solu-Medrol®, a more specific diabetes covariate coding, a covariate coding for post-transplantation time after 3 months, and screening for influence of CYP enzymes and P-gp transporters may be needed to improve upon the developed model.

4.3 THE FINAL MODEL FOR CYCLOSPORIN A

After a preliminary screening of different models, the 2-compartment model with lagtime showed the most promise. Though stability problems had to be overcome, the model gave a good fit and had an acceptable run time. Run time was one important criterion, as this model would be implemented in a clinical study. Validation of the model would also be less cumbersome, and less computational power would be needed to run the model in NONMEM. Possibly by increasing computational power other models might have proven to be useful as well, but this was not tested. The selected model was tested rigorously, before inclusion of covariates.

For the final model there is an even distribution in the scatter plots of weighted residuals, which is acceptable.

The goodness of fit scatter plots of the final model shown in figure 11 shows a good correlation between observed and predicted concentrations, which indicates that the model fits the observed concentrations. However, it can be seen that the model does have problems in predicting lower concentrations. A slight over-prediction for low level concentration is observable, but is considerably improved in comparison to earlier models during screening. This is also evident when reviewing the individual fits (Appendix 7.6). Under-prediction for higher concentrations can be seen for some patients, in particular around peak concentrations. Prediction problems were somewhat addressed by the inclusion of covariates, but none the less more elaborate models and larger data sets may be needed to improve the model.

One problem that was not resolved was the high degree of under-prediction for concentrations observed beyond 2000 hours (~12 weeks). As can be seen on the quality of fit plots (Appendix 7.5), there are four patients that have observations after 2000 hours. Patient no. 8 provides observations up to ~2200 hours, patient no. 6 and 9 provide observations up to ~2500 hours, and patient no. 10 provides observations up to 3000 hours. There are too few observations for the model to correctly predict concentrations at this point, when compared to observations before 2000 hours. Individual predictions are more accurate than population predicted concentrations, which is to be expected. As mentioned in paragraph 2.1.1 Saint-Marcoux et al. [26] coded the post-transplantation time in three intervals of < 2 weeks, between 2 weeks and three months, and > 3 months. A more appropriate covariate coding could have corrected this under-prediction, but this theory was not tested.

Another point that should be mentioned is that the model incorporates data from directly after the transplantation, while in the clinical setting of the proposed add-on study patients will not be available for the study until approximately one week post-transplantation. During the first days after transplantation patients are in intensive care. Highly variable CsA concentrations were observed during this first week in the SUPER-CsA [43] data available for model development. The high degree of variability may possibly influence the model in negative way that may be seen once the model is applied in a clinical setting. Some possible reasons for this variability have been discussed in paragraph 4.2. The limitations caused by the first week post-transplantation were not fully explored in this thesis.

4.4 VALIDATING THE MODEL

Validation can be viewed as an objective evaluation of the predictability of a model, and assess whether deficiencies of the model will have a noticeable effect. The validation process will also evaluate the clinical applicability of the model. In literature the different methods for validation is discussed, but one cannot conclude which method that is best. It is still an evolving science, and it is the personal preference of the analyst that usually decides the choice. Justification for any choice should be given [5, 6, 63].

The technique used to examine the predictive performance of the model was recommended by the Food and Drug Administration. The data-splitting gave very variable OFV and clearly showed that the model has a lack of robustness. The difference between the maximum and minimum value were 253.09, which is significantly more than the maximum allowed value of < 3.84. Parameter values however were very consistent for the developed model. No significant deviation can be seen in the estimated parameter values for the 10 subsets.

The data set used to develop this model was different from most other data set used previously, by including a large amount of data before and after the 12-hours PK-profiling. This meant that while performing predictive performance check, the observed concentrations chosen to be included in the subset for the remaining ~10 % could not be chosen as other studies have done. Usually the observed concentrations included are C_0, C_1, C_2 and/or C_3, and the predictive performance is evaluated from AUC_{0-4} or AUC_{0-12} calculations [50, 64]. This could not be done for the data set used in this model because there were 11 patients (SUPER-CsA patient no. 1-6, 13, 15-17 and 20 [43]) that did not have PK-profiling performed at all, only routine follow-up concentrations. Therefore the observed concentrations included were just the first and/or the second concentration for that specific patient. Predictive performance was then compared by calculations of the individual predicted concentrations. AUC_{0-12} was not used in this case. Results showed that precision were higher when “no observed concentrations” and “the first and second observed concentrations” were used, than when only the “first observed concentration” was included in the input file. This may seem unusual, but upon reviewing the observed concentrations that are included, it is evident that the first concentration is a highly variable concentration measured quite recent after the transplantation. This is especially the case for the SUPER-CsA [43] patients that provided much more data. As mentioned earlier, this time period is

plagued by many factors that might cause a high degree of inter-individual and residual variability. The patients undergo many physiological changes that may affect the overall CsA concentrations and effects. Another point that might have affected the precision in this validation test is the fact that C_0 and C_2 measurements were not differentiated. The first observed concentrations that were included in the input file for validation was always a C_0 measurement for the MIMPARA [45] patients and the patients from the age effect study [44]. The SUPER-CsA [43] patient provided first observed concentrations that could be C_0 or C_2. A small absolute estimation error for a C_0 measurement will affect the overall precision for the entire group in a larger degree than the same absolute estimation error for a C_2 measurement, but this is clinical reality. Inclusion of two concentration data points will improve the precision of the model on the basis that the model has more data to build upon.

The Jackknife technique for acquiring 95 % CI was preferred over bootstrapping because it required less computation. The 49 Jackknife runs required approximately 48 hours to complete. The result was acceptable, though the lack of robustness of the model may have affected this estimation. This is evident as patient no. 2, 16, 20, 33, 34, 65 and 69 gave very high estimates of V_P//F, which were close to the upper level range for starting estimates of this parameter (Appendix 7.7). This was a problem with the model from the beginning. Lack of robustness meant that the model parameter estimates could easily shoot up to high levels that seemed unrealistic. The final models variable parameter estimates which gave a mean value for V_P//F of 351.5 L and maximum value of 399 L is acceptable, but seem high when compared to other studies such as Rousseau et al. [27], and Saint-Marcoux et al. [26] which had 133 L and 129.4 L, respectively.

External validation of the model did show the same tendencies as for the internal validation. Inclusion of the first observed concentration showed a large deviation when compared to inclusion of none or more observed concentrations. This anomaly has been pointed out earlier when discussing the internal validation. Overall the results were as expected, with improvements in bias and precision, when more observed concentrations are included in the data set.

The biggest problem of the developed model was discovered during validation. The lack of robustness caused deterioration of all the estimated values. This was a problem that occurred after inclusion of the significant covariates. Though the covariates proved to be statistically

significant, it is possible that the clinical significance of their effect is overestimated. The criteria set up for inclusion of covariates in to the final model, will need revision to possibly only keep covariates of the greatest strength for the final model. As the new criteria will be stricter this approach will not interfere with statistical significance as defined by the maximum likelihood approach used by NONMEM.

5 CONCLUSIONS

By using the non-linear mixed-effect modelling program NONMEM a pharmacokinetic population model was developed for renal transplant patients receiving cyclosporine A. This model may in future settings be used to design dosage regimens for other patients. Optimization of therapeutic drug monitoring in renal transplant patients receiving cyclosporine A was the overall goal of the work of this thesis.

Development of the pharmacokinetic population model was done in a stepwise manner. A screening process where different compartment models were tested with different absorption profiles eliminated all but one model, which was chosen for further development. The 2-compartment model with an absorption lagtime gave the best fit with acceptable run time for the cyclosporin A data.

The 2-compartment model with an absorption lagtime was improved upon by adding patient specific covariates. These covariates were added to explain the inter-individual variations seen in the pharmacokinetic parameters. The large amount of data used produced many significant covariates, but only the most significant were chosen for inclusion in the model. The covariates that gave the most significant impact on the model were the time-dependent covariates steroid dose (mg) and post-transplantation time (weeks). By adding these covariates the error in predicted concentrations in the first 2 weeks after transplantation was significantly less. However many covariates gave very variable estimates, which may indicate that they have an overestimated clinical effect, and could possibly be removed from the model. A stricter screening procedure for covariates may need to be implemented.

Results from the internal validation performed showed that the model had a lack of robustness. This may possibly be caused by the large amount of covariates added to the model. The model is not able to deal effectively with changes in start estimates for the pharmacokinetic parameters. However results do show that the model describes the cyclosporin A data in a good manner. More external testing may be needed to prove the appropriateness and the effectiveness of the model, as external validation is the strongest evidence for a good fit.

Further refinement may be needed to improve upon the stability of the model, but this model does provide a good basis upon which a dosage regimen for cyclosporin A may be designed and tested clinically.

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7 APPENDIX

7.1 PARTIAL INPUT FILE FOR NONMEM

ID	AMT	RATE	DAT1=DROP	TIME	C=DV	WT	UREA	SCR	CRCL	MDV	SS	II	CMT	FLAG	AGE	GENDER	HEIGHT	TXT	STER	BMI	3A5	LBM
1	325000	0	10.06.2005	6	0	66	25,7	403	22,3	1	2	12	1	1	56	2	1,74	0	80	21,8	3	48,98
1	0	0	10.06.2005	8	0	66	25,7	403	22,3	1	0	0	2	1	56	2	1,74	0	80	21,8	3	48,98
1	325000	0	10.06.2005	20	0	66	25,7	403	22,3	1	0	0	1	1	56	2	1,74	0	80	21,8	3	48,98
1	0	0	10.06.2005	22	0	66	25,7	403	22,3	1	0	0	2	1	56	2	1,74	0	80	21,8	3	48,98
1	325000	0	11.06.2005	8	0	67	15,3	154	54,6	1	0	0	1	1	56	2	1,74	0	80	22,1	3	49,27
1	0	0	11.06.2005	8	480	67	15,3	154	54,6	0	0	0	2	1	56	2	1,74	0	80	22,1	3	49,27
1	325000	0	11.06.2005	20	0	67	15,3	154	54,6	1	0	0	1	1	56	2	1,74	0	80	22,1	3	49,27
1	0	0	11.06.2005	22	0	67	15,3	154	54,6	1	0	0	2	1	56	2	1,74	0	80	22,1	3	49,27
1	325000	0	12.06.2005	6	0	67,5	10,2	80	97,5	1	0	0	1	1	56	2	1,74	0	80	22,3	3	49,42
1	0	0	12.06.2005	8	0	67,5	10,2	80	97,5	1	0	0	2	1	56	2	1,74	0	80	22,3	3	49,42
1	325000	0	12.06.2005	20	0	67,5	10,2	80	97,5	1	0	0	1	1	56	2	1,74	0	80	22,3	3	49,42
1	0	0	12.06.2005	22	0	67,5	10,2	80	97,5	1	0	0	2	1	56	2	1,74	0	80	22,3	3	49,42
1	325000	0	13.06.2005	6	0	67,3	8,1	54	138,8	1	0	0	1	1	56	2	1,74	0	70	22,2	3	49,36
1	0	0	13.06.2005	8	1580	67,3	8,1	54	138,8	0	0	0	2	1	56	2	1,74	0	70	22,2	3	49,36
1	325000	0	13.06.2005	20	0	67,3	8,1	54	138,8	1	0	0	1	1	56	2	1,74	0	70	22,2	3	49,36
1	0	0	13.06.2005	22	0	67,3	8,1	54	138,8	1	0	0	2	1	56	2	1,74	0	70	22,2	3	49,36
1	325000	0	14.06.2005	6	0	67,7	7,7	51	146,4	1	0	0	1	1	56	2	1,74	0	60	22,4	3	49,48
1	0	0	14.06.2005	8	0	67,7	7,7	51	146,4	1	0	0	2	1	56	2	1,74	0	60	22,4	3	49,48
1	325000	0	14.06.2005	20	0	67,7	7,7	51	146,4	1	0	0	1	1	56	2	1,74	0	60	22,4	3	49,48
1	0	0	14.06.2005	22	0	67,7	7,7	51	146,4	1	0	0	2	1	56	2	1,74	0	60	22,4	3	49,48
1	325000	0	15.06.2005	6	0	67	8,7	56	134	1	0	0	1	1	56	2	1,74	0	50	22,1	3	49,27
1	0	0	15.06.2005	8	1840	67	8,7	56	134	0	0	0	2	1	56	2	1,74	0	50	22,1	3	49,27
1	325000	0	15.06.2005	20	0	67	8,7	56	134	1	0	0	1	1	56	2	1,74	0	50	22,1	3	49,27
1	0	0	15.06.2005	22	0	67	8,7	56	134	1	0	0	2	1	56	2	1,74	0	50	22,1	3	49,27
1	325000	0	16.06.2005	6	0	66	9,5	56	133,4	1	0	0	1	1	56	2	1,74	0	40	21,8	3	48,98
1	0	0	16.06.2005	8	0	66	9,5	56	133,4	1	0	0	2	1	56	2	1,74	0	40	21,8	3	48,98
1	325000	0	16.06.2005	20	0	66	9,5	56	133,4	1	0	0	1	1	56	2	1,74	0	40	21,8	3	48,98
1	0	0	16.06.2005	22	0	66	9,5	56	133,4	1	0	0	2	1	56	2	1,74	0	40	21,8	3	48,98
~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
11	200000	0	19.09.2005	6	0	73,8	11,5	110	80,3	1	0	0	1	1	52	1	1,88	4	30	20,9	3	58,47
11	0	0	19.09.2005	8	1270	73,8	11,5	110	80,3	0	0	0	2	1	52	1	1,88	4	30	20,9	3	58,47
11	200000	0	19.09.2005	20	0	73,8	11,5	110	80,3	1	0	0	1	1	52	1	1,88	4	30	20,9	3	58,47
11	0	0	19.09.2005	22	0	73,8	11,5	110	80,3	1	0	0	2	1	52	1	1,88	4	30	20,9	3	58,47
11	200000	0	20.09.2005	6	0	73,8	11,5	110	80,3	1	0	0	1	1	52	1	1,88	4	30	20,9	3	58,47
11	0	0	20.09.2005	8	0	73,8	11,5	110	80,3	1	0	0	2	1	52	1	1,88	4	30	20,9	3	58,47
11	200000	0	20.09.2005	22	0	73,8	11,5	110	80,3	1	0	0	1	1	52	1	1,88	4	30	20,9	3	58,47
11	0	0	21.09.2005	7,75	202	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	200000	0	21.09.2005	7,8	0	75,3	10,3	110	81,3	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	8,1	240	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	8,37	607	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	8,8	1633	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96

11	0	0	21.09.2005	9,32	1803	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	9,83	1369	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	10,77	1538	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	11,75	1286	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	13,77	519	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	15,67	377	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	17,75	276	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	21.09.2005	19,55	235	75,3	10,3	110	81,3	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	175000	0	21.09.2005	20,5	0	75,3	10,3	110	81,3	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	22.09.2005	7,65	238	75,3	9,9	113	79,9	0	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	200000	0	22.09.2005	7,66	0	75,3	9,9	113	79,9	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	22.09.2005	8	0	75,3	9,9	113	79,9	1	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	175000	0	22.09.2005	20	0	75,3	9,9	113	79,9	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	22.09.2005	22	0	75,3	9,9	113	79,9	1	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	200000	0	23.09.2005	6	0	75,3	9,9	113	79,9	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	23.09.2005	8	0	75,3	9,9	113	79,9	1	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	175000	0	23.09.2005	20	0	75,3	9,9	113	79,9	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	23.09.2005	22	0	75,3	9,9	113	79,9	1	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	200000	0	24.09.2005	6	0	75,3	9,9	113	79,9	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	24.09.2005	8	0	75,3	9,9	113	79,9	1	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
11	175000	0	24.09.2005	20	0	75,3	9,9	113	79,9	1	0	0	1	1	52	1	1,88	4	30	21,3	3	58,96
11	0	0	24.09.2005	22	0	75,3	9,9	113	79,9	1	0	0	2	1	52	1	1,88	4	30	21,3	3	58,96
~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
33	175000	0		0	0	78			69,4	1	2	12	1	1	68	2	1,56	3	20	32,1	3	45
33	0	0		11,5	340	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	175000	0		11,7	0	78			69,4	1	0	0	1	1	68	2	1,56	3	20	32,1	3	45
33	0	0		12,2	1260	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		12,7	2492	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		13,23	2046	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		13,7	1415	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		14,25	1413	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		14,72	1217	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		15,67	787	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		16,72	739	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		17,7	585	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		19,75	543	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		21,7	487	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
33	0	0		23,23	404	78			69,4	0	0	0	2	1	68	2	1,56	3	20	32,1	3	45
~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
70	100000	0		0	0	49			109,3	1	2	12	1	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0		11,82	118	49			109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	125000	0		11,88	0	49			109,3	1	0	0	1	1	73,8	2	1,59	7,3	15	19,4	1	37,68

70	0	0	12,15	296	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	12,38	1285	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	12,9	1677	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	13,38	1308	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	13,85	867	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	14,93	637	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	15,86	412	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	18,01	286	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	19,83	217	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	21,81	206	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
70	0	0	23,4	139	49	109,3	0	0	0	2	1	73,8	2	1,59	7,3	15	19,4	1	37,68
~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~

7.2 CONTROL FILES FOR NONMEM

7.2.1 1-compartment model with lagtime

```
$PROBLEM ORAL ADMIN., 1 COMP., 1. ORDER ABS., WITH ABS. LAG TIME; Created by Truc Van Le

$DATA Input.ALL.DATA.txt

$INPUT ID AMT RATE DAT1=DROP TIME C=DV WT UREA=DROP SCR=DROP CRCL MDV SS=DROP II=DROP CMT=DROP
FLAG AGE GEN HGHT TXT STER BMI C3A5 LBM

$SUBROUTINE ADVAN2 TRANS2

$PK
   CL=THETA(1)*EXP(ETA(1))         ;Clearance (CL/F) L/hr
   V=THETA(2)*EXP(ETA(2))          ;Volume (V/F), L
   KA=THETA(3)*EXP(ETA(3))         ;Absorption rate constant, 1/hr
   ALAG1=THETA(4)*EXP(ETA(4))      ;Absorption lag time, hr

   S2=V

$ERROR
   IPRED=F
   Y=F+F*ERR(1)+ERR(2)

$THETA (1,23,40)          ;THETA(1) is POPCL/F
$THETA (10,144,200)       ;THETA(2) is POPV/F
$THETA (0.1,4.81)         ;THETA(3) is POPKA
$THETA (0.1,0.454,0.7)    ;THETA(4) is POPLAGTIME

$OMEGA BLOCK (3)
0.0901                    ;BSVCL/F
0.0788 0.147              ;BSVV/F
-0.154 -0.107  0.29       ;BSVKA

$OMEGA
0.0111                    ;BSVALAG1

$SIGMA 0.102  ;ERRCV, Proportional error (%)
$SIGMA 7.35   ;ERRSD, Additive error (ng/ml)

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$COVARIANCE

$TABLE ID TIME DV IPRED MDV
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID CL V KA ALAG1 ETA1 ETA2 ETA3 ETA4 WT CRCL FLAG AGE GEN HGHT TXT STER BMI C3A5 LBM
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

7.2.2 2-compartment model with lagtime

```
$PROBLEM ORAL ADMIN., 2 COMP., 1. ORDER ABS., WITH ABS. LAGTIME; Created by Truc Van Le

$DATA Input.FINAL.FULL.MODEL.txt

$INPUT ID AMT RATE DAT1=DROP TIME C=DV WT UREA=DROP SCR=DROP CRCL MDV SS II CMT FLAG AGE GEN HGHT
TXT STER BMI C3A5=DROP LBM

$SUBROUTINE ADVAN4

$PK
   TVCL=THETA(1)
   TVV1=THETA(2)
   TVQ=THETA(3)
   TVV2=THETA(4)
   TVKA=THETA(5)
   TVALAG=THETA(6)

   CL=TVCL*EXP(ETA(1))                  ;Clearance (CL/F) L/hr
   V1=TVV1*EXP(ETA(2))                  ;Central volume (V1/F), L
   Q=TVQ*EXP(ETA(3))                    ;Intercompartmental clearance (Q/F)
   V2=TVV2*EXP(ETA(4))                  ;Peripheral volume (V2/F), L
   KA=TVKA*EXP(ETA(5))                  ;Absorption rate constant, 1/hr
   ALAG1=TVALAG*EXP(ETA(6))             ;Absorption lag time, hr

   S2=V1

   K=CL/V1
   K23=Q/V1
   K32=Q/V2

$ERROR
   IPRED=F
   Y=F+F*ERR(1)+ERR(2)

$THETA (23,26.1,40)      ;THETA(1) is POPCL/F
$THETA (75,77.9,95)      ;THETA(2) is POPV1/F
$THETA (15,20.4,30)      ;THETA(3) is POPQ/F
$THETA (320,342,400)     ;THETA(4) is POPV2/F
$THETA (.5,1.86,3)       ;THETA(5) is POPKA
$THETA (0.2,0.444,0.7)   ;THETA(6) is POPLAGTIME

$OMEGA
0.07     ;BSVCL/F
0.2      ;BSVV1/F
0.1      ;BSVQ/F
2.35     ;BSVV2/F
0.315    ;BSVKA
0.0085   ;BSVALAG1

$SIGMA 0.0662    ;ERRCV, Proportional error (%)
$SIGMA 24.4      ;ERRSD, Additive error (ng/ml)

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$COVARIANCE

$TABLE ID TIME DV IPRED MDV
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID CL V1 Q V2 KA ALAG1 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 WT CRCL FLAG AGE GEN HGHT TXT STER
BMI LBM
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

7.2.3 2-compartment model with zero order absorption

```
$PROBLEM ORAL ADMIN., 2 COMP., 0. ORDER ABS., WITH LAGTIME; Created by Truc Van Le

$DATA Input.ALL.DATA.txt

$INPUT ID AMT RATE DAT1=DROP TIME C=DV WT UREA=DROP SCR=DROP CRCL MDV SS=DROP II=DROP CMT=DROP
FLAG AGE GEN HGHT TXT STER BMI C3A5 LBM

$SUBROUTINE ADVAN3

$PK
   CL=THETA(1)*EXP(ETA(1))        ;Clearance (CL/F) L/hr
   V1=THETA(2)*EXP(ETA(2))        ;Central volume (V1/F), L
   Q=THETA(3)*EXP(ETA(3))         ;Intercompartmental clearance (Q/F), L/hr
   V2=THETA(4)*EXP(ETA(4))        ;Peripheral volume (V2/F), L
   KA=THETA(5)*EXP(ETA(5))        ;Absorption rate constant, 1/hr
   ALAG1=THETA(6)*EXP(ETA(6))     ;Absorption lag time, hr

   S2=V1

   K=CL/V1
   K12=Q/V1
   K21=Q/V2

$ERROR
   IPRED=F
   Y=F+F*ERR(1)+ERR(2)

$THETA (1,22.1)          ;THETA(1) is POPCL/F
$THETA (1,1.15)          ;THETA(2) is POPV1/F
$THETA (1,186)           ;THETA(3) is POPQ/F
$THETA (10,120)          ;THETA(4) is POPV2/F
$THETA (0.1,0.32)        ;THETA(5) is POPKA
$THETA (0.1,0.33)        ;THETA(6) is POPLAGTIME

$OMEGA BLOCK (4)
0.184                                                ;BSVCL/F
0.239    0.376                                       ;BSVV1/F
-0.445   -0.578   1.08                               ;BSVQ/F
-0.00206 -0.00424 0.00686  0.00764                   ;BSVV2/F

$OMEGA
0.01     ;BSVKA
0.000003 ;BSVALAG1

$SIGMA 0.122  ;ERRCV, Proportional error (%)
$SIGMA 200    ;ERRSD, Additive error (ng/ml)

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$COVARIANCE

$TABLE ID TIME DV IPRED MDV
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID CL V1 Q V2 KA ALAG1 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 WT CRCL FLAG AGE GEN HGHT TXT STER
BMI C3A5 LBM
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

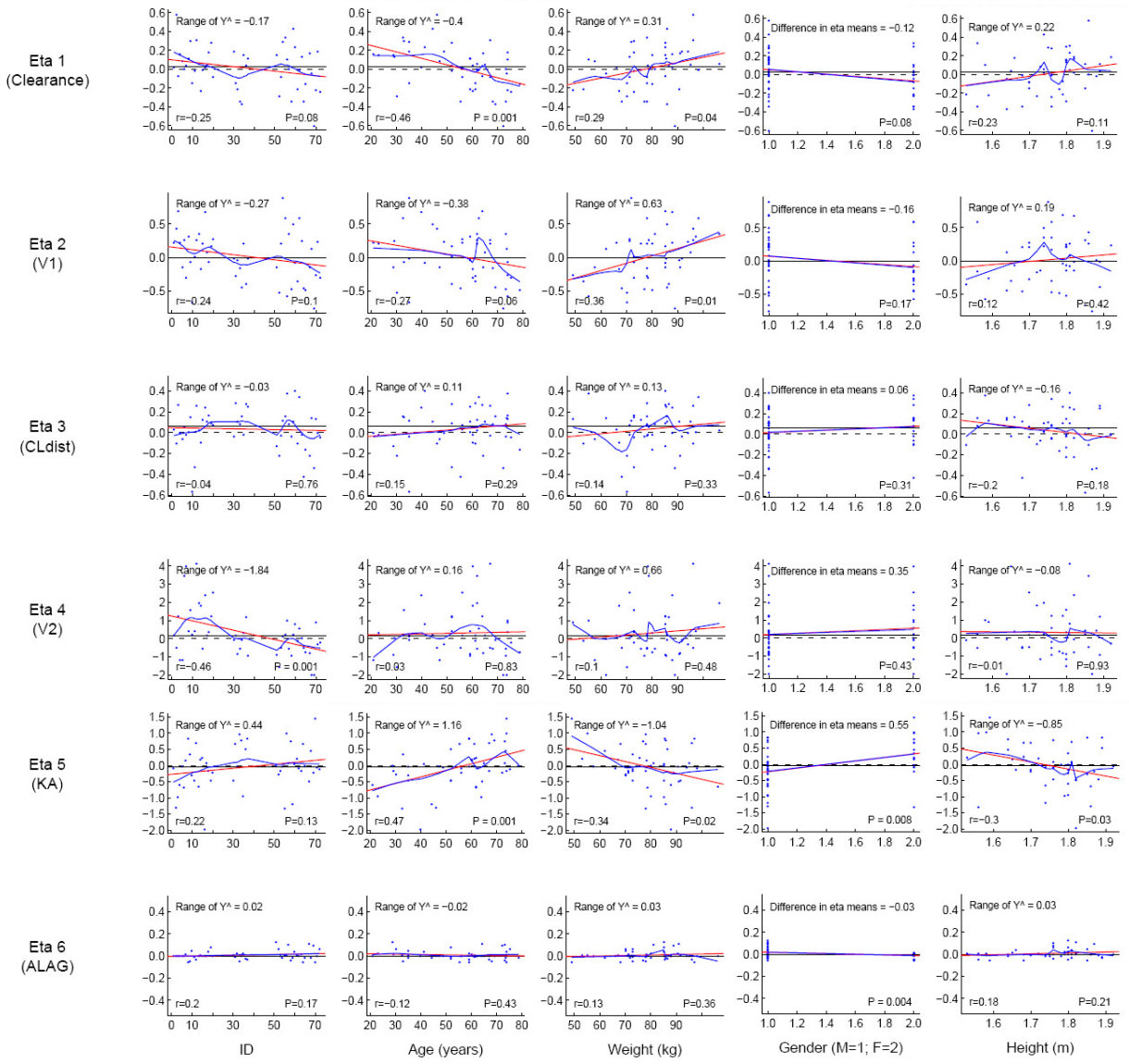
7.2.4 2-compartment model with 4 Erlang compartments

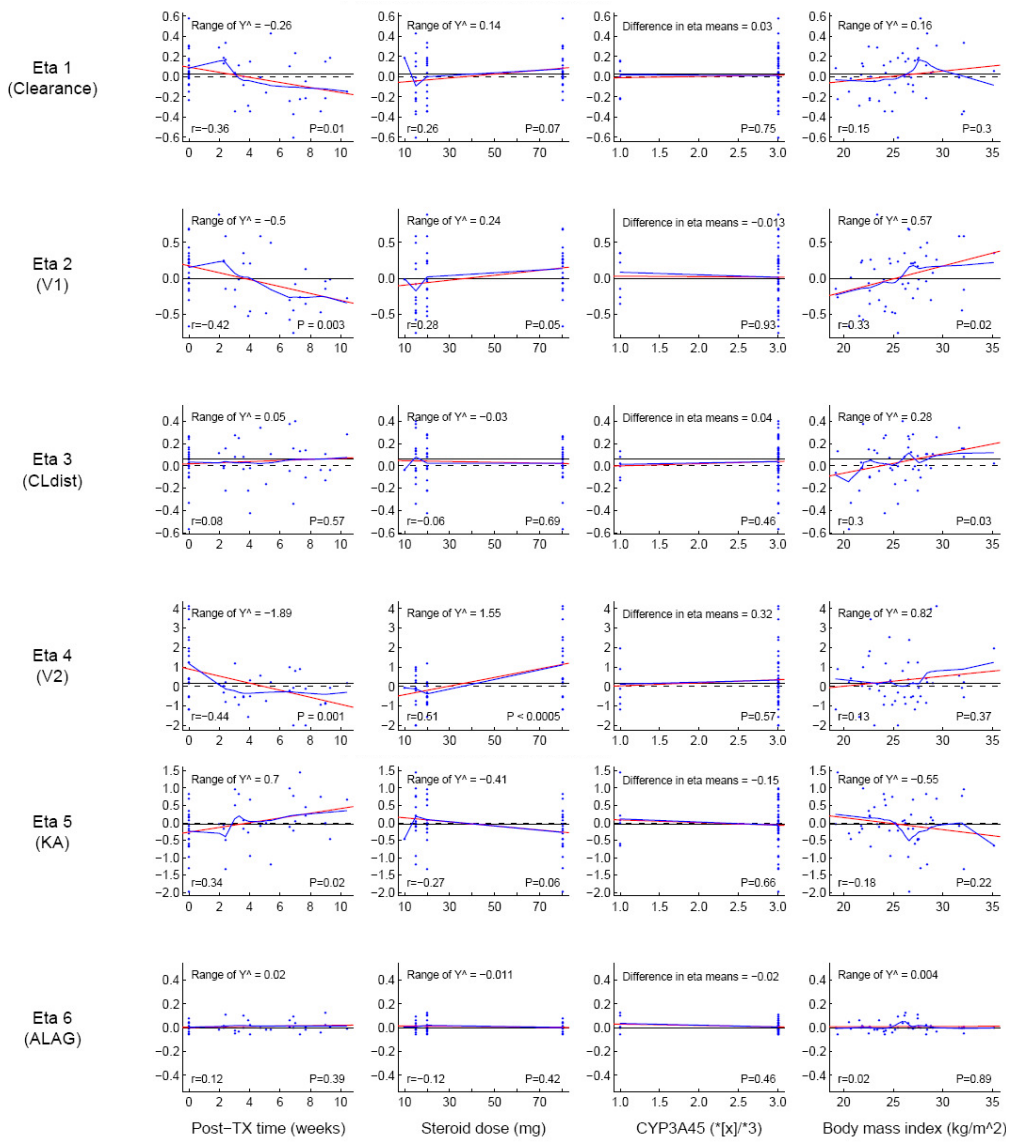
```
$PROBLEM ORAL ADMIN., 2 COMP., ERLANG DISTR.; Created by Truc Van Le

$DATA Input.ALL.DATA.txt

$INPUT ID AMT RATE DAT1=DROP TIME C=DV WT UREA=DROP SCR=DROP CRCL MDV SS=DROP II=DROP CMT=DROP
FLAG AGE GEN HGHT TXT STER BMI C3A5 LBM

$SUBROUTINE ADVAN5 SS5

$MODEL COMP=(DEPOT,DEFDOSE)
       COMP=(DELA)
       COMP=(DELA2)
       COMP=(DELA3)
       COMP=(DELA4)
       COMP=(CENTRAL,DEFOBS)
       COMP=(PERIPH)

$PK
   K12=THETA(1)*EXP(ETA(1))

   K23=K12
   K34=K12
   K45=K12
   K56=K12

   TVCL=THETA(2)
   TVV6=THETA(3)
   TVV7=THETA(4)
   TVQ=THETA(5)

   CL=TVCL*EXP(ETA(2))        ;Clearance (CL/F), L/hr
   V6=TVV6*EXP(ETA(3))        ;Central volume (V6/F), L
   V7=TVV7*EXP(ETA(4))        ;Peripheral volume (V7/F), L
   Q=TVQ*EXP(ETA(5))          ;Intercompartmental clearance (Q/F), L

   S6=V6

   K60=CL/V6
   K67=Q/V6
   K76=Q/V7

$ERROR
   IPRED=F
   Y=F+F*ERR(1)+ERR(2)

$THETA (0.1,7.84)         ;THETA(1) is POPLAG, K12
$THETA (10,28.1)          ;THETA(2) is POPCL/F
$THETA (10,58.5)          ;THETA(3) is POPV6/F
$THETA (10,215,1000)      ;THETA(4) is POPV7/F
$THETA (10,23.1)          ;THETA(5) is POPQ/F

$OMEGA
0.06                      ;BSVK12/F
0.09                      ;BSVCL/F
0.27                      ;BSVV6/F
0.91                      ;BSVV7/F
0.02                      ;BSVQ/F

$SIGMA 0.02   ;ERRCV, Proportional error (%)
$SIGMA 1370   ;ERRSD, Additive error (ng/ml)

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$COVARIANCE

$TABLE ID TIME IPRED MDV
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID K12 CL V6 V7 Q ETA1 ETA2 ETA3 ETA4 ETA5
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

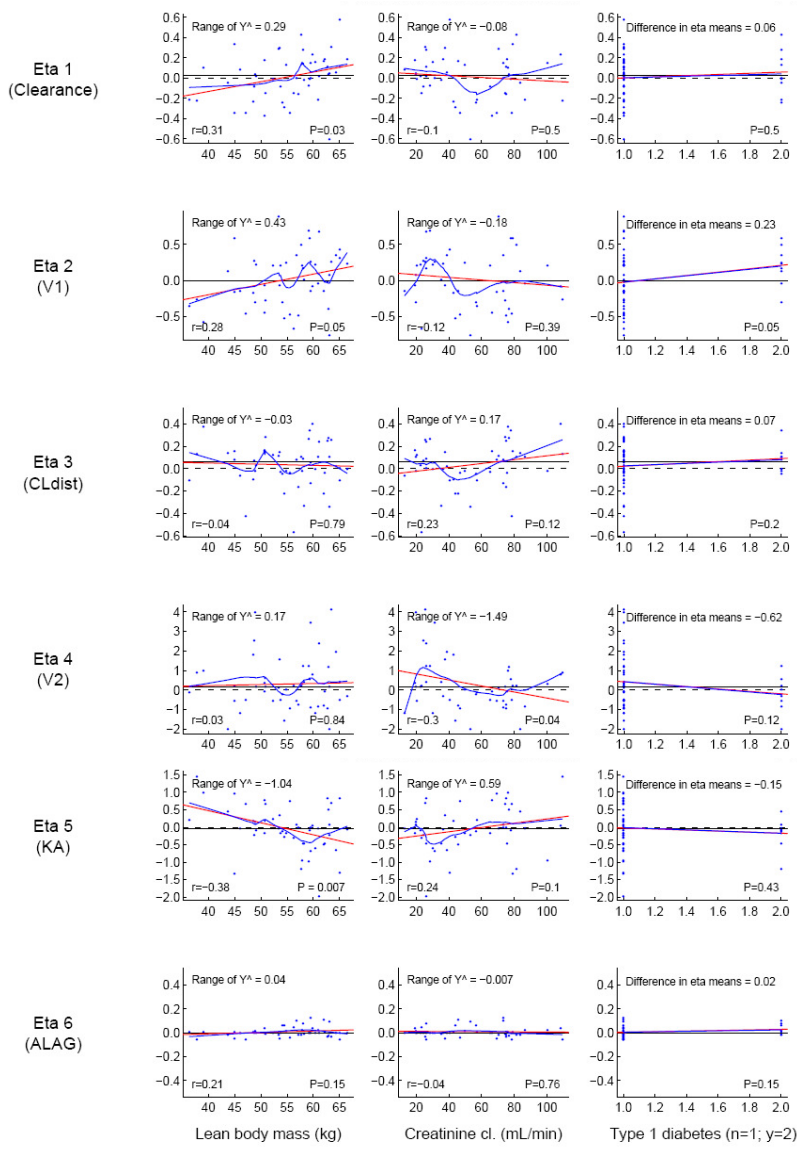
7.2.5 3-compartment model with lagtime

```
$PROBLEM ORAL ADMIN., 3 COMP., 1. ORDER ABS., WITH LAGTIME; Created by Truc Van Le

$DATA Input.ALL.DATA.txt

$INPUT ID AMT RATE DAT1=DROP TIME C=DV WT UREA=DROP SCR=DROP CRCL MDV SS=DROP II=DROP CMT=DROP
FLAG AGE GEN HGHT TXT STER BMI C3A5 LBM

$SUBROUTINE ADVAN12

$PK
   NCMT=3
   CL=THETA(1)*EXP(ETA(1))         ;Clearance (CL/F), L/hr
   V1=THETA(2)*EXP(ETA(2))         ;Central volume (V1/F), L
   CLRA=THETA(3)*EXP(ETA(3))       ;Intercompartmental clearance,
                                    central+peripheral 1 (CLRA/F), L/hr
   V2=THETA(4)*EXP(ETA(4))         ;Peripheral volume 1 (V2/F), L
   CLSL=THETA(5)*EXP(ETA(5))       ;Intercompartmental clearance,
                                    central+peripheral 2 (CLSL/F), L/hr
   V3=THETA(6)*EXP(ETA(6))         ;Perpheral volume 2 (V3/F), L
   KA=THETA(7)*EXP(ETA(7))         ;Absorption rate constant, 1/hr
   ALAG1=THETA(8)*EXP(ETA(8))      ;Absorption lag time, hr

   S2=V1

   K=CL/V1
   K23=CLRA/V1
   K24=CLSL/V1
   K32=CLRA/V2
   K42=CLSL/V3

$ERROR
   IPRED=F
   Y=F+F*ERR(1)+ERR(2)

$THETA (10,23.7)          ;THETA(1) is POPCL/F
$THETA (10,50.6)          ;THETA(2) is POPV1/F
$THETA (10,10.1)          ;THETA(3) is POPCLRA/F
$THETA (10,54.6)          ;THETA(4) is POPV2/F
$THETA (1,21.3)           ;THETA(5) is POPCLSL/F
$THETA (10,187)           ;THETA(6) is POPV3/F
$THETA (1,1.2)            ;THETA(7) is POPKA
$THETA (0.1,0.447)        ;THETA(8) is POPLAGTIME

$OMEGA BLOCK(6)
0.0729                                                  ;BSVCL/F
0.001    0.87                                           ;BSVV1/F
0.001    0.001    0.162                                 ;BSVCLRA/F
0.001    0.001    0.001    0.69                         ;BSVV2/F
0.001    0.001    0.001    0.001    0.114               ;BSVCLSL/F
0.001    0.001    0.001    0.001    0.001    0.51       ;BSVV3/F

$OMEGA
0.00696  ;BSVKA
0.000851 ;BSVALAG1

$SIGMA 0.0633 ;ERRCV, Proportional error (%)
$SIGMA 123    ;ERRSD, Additive error (ng/ml)

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC NOABORT

$COVARIANCE

$TABLE ID TIME DV IPRED MDV NCMT
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID CL V1 CLRA V2 CLSL V3 KA ALAG1 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 WT CRCL FLAG AGE
GEN HGHT TXT STER BMI C3A5 LBM
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

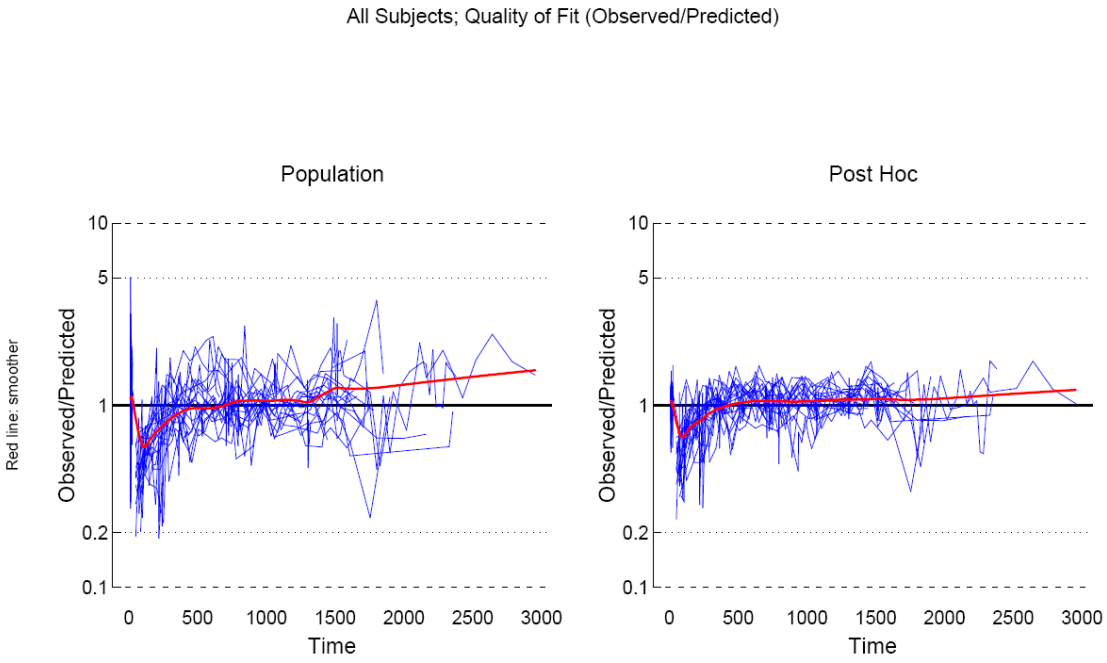
7.3 PLOTS OF POST HOC ETAS VS. COVARIATES OF START MODEL



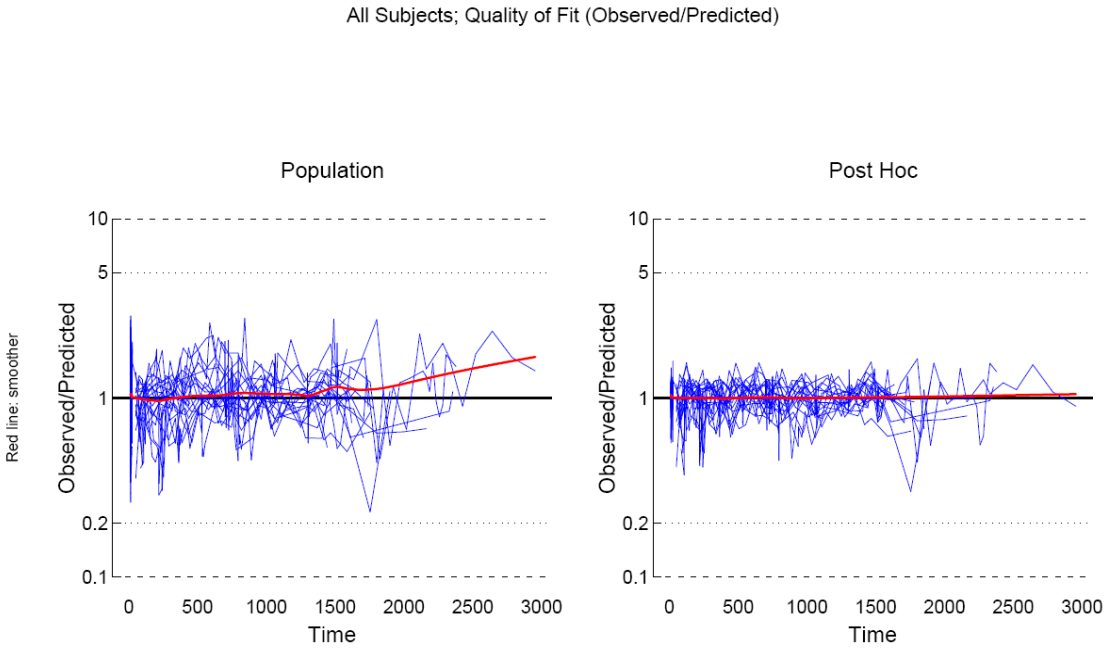

Eta 1 (Clearance)
Eta 2 (V1)
Eta 3 (CLdist)
Eta 4 (V2)
Eta 5 (KA)
Eta 6 (ALAG)
Range of Y^ = −0.26
r=−0.36
P=0.01
Range of Y^ = 0.14
r=0.26
P=0.07
Difference in eta means = 0.03
P=0.75
Range of Y^ = 0.16
r=0.15
P=0.3
Range of Y^ = −0.5
r=−0.42
P = 0.003
Range of Y^ = 0.24
r=0.28
P=0.05
Difference in eta means = −0.013
P=0.93
Range of Y^ = 0.57
r=0.33
P=0.02
Range of Y^ = 0.05
r=0.08
P=0.57
Range of Y^ = −0.03
r=−0.06
P=0.69
Difference in eta means = 0.04
P=0.46
Range of Y^ = 0.28
r=0.3
P=0.03
Range of Y^ = −1.89
r=−0.44
P = 0.001
Range of Y^ = 1.55
r=0.61
P < 0.0005
Difference in eta means = 0.32
P=0.57
Range of Y^ = 0.82
r=0.13
P=0.37
Range of Y^ = 0.7
r=0.34
P=0.02
Range of Y^ = −0.41
r=−0.27
P=0.06
Difference in eta means = −0.15
P=0.66
Range of Y^ = −0.55
r=−0.18
P=0.22
Range of Y^ = 0.02
r=0.12
P=0.39
Range of Y^ = −0.011
r=−0.12
P=0.42
Difference in eta means = −0.02
P=0.46
Range of Y^ = 0.004
r=0.02
P=0.89
Post−TX time (weeks)
Steroid dose (mg)
CYP3A45 (*[x]/*3)
Body mass index (kg/m^2)


Eta 1
(Clearance)
Range of Y^ = 0.29
r=0.31
P=0.03
Range of Y^ = −0.08
r=−0.1
P=0.5
Difference in eta means = 0.06
P=0.5
Eta 2
(V1)
Range of Y^ = 0.43
r=0.28
P=0.05
Range of Y^ = −0.18
r=−0.12
P=0.39
Difference in eta means = 0.23
P=0.05
Eta 3
(CLdist)
Range of Y^ = −0.03
r=−0.04
P=0.79
Range of Y^ = 0.17
r=0.23
P=0.12
Difference in eta means = 0.07
P=0.2
Eta 4
(V2)
Range of Y^ = 0.17
r=0.03
P=0.84
Range of Y^ = −1.49
r=−0.3
P=0.04
Difference in eta means = −0.62
P=0.12
Eta 5
(KA)
Range of Y^ = −1.04
r=−0.38
P = 0.007
Range of Y^ = 0.59
r=0.24
P=0.1
Difference in eta means = −0.15
P=0.43
Eta 6
(ALAG)
Range of Y^ = 0.04
r=0.21
P=0.15
Range of Y^ = −0.007
r=−0.04
P=0.76
Difference in eta means = 0.02
P=0.15
Lean body mass (kg)
Creatinine cl. (mL/min)
Type 1 diabetes (n=1; y=2)

7.4 QUALITY OF FIT PLOT OF THE START MODEL

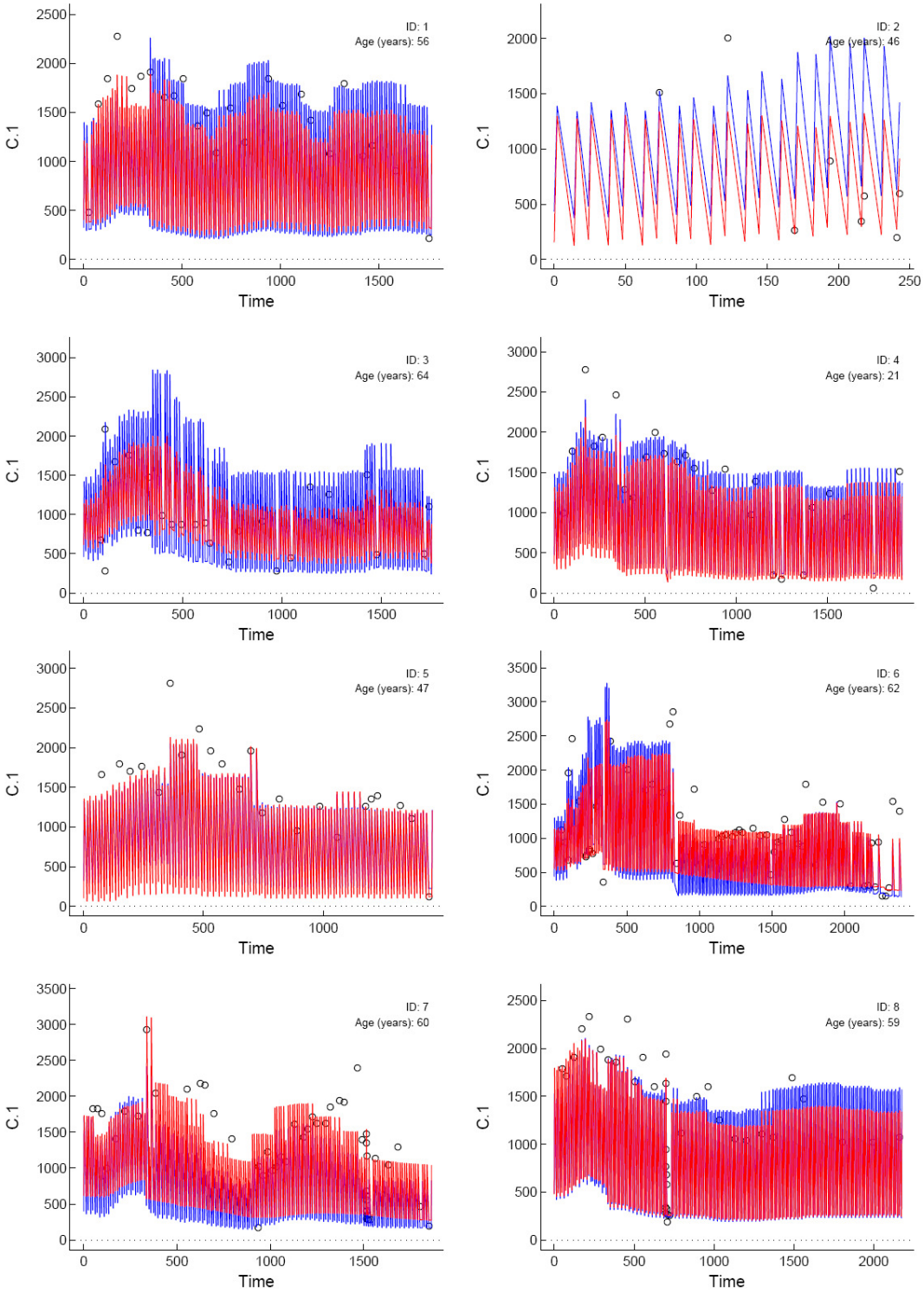


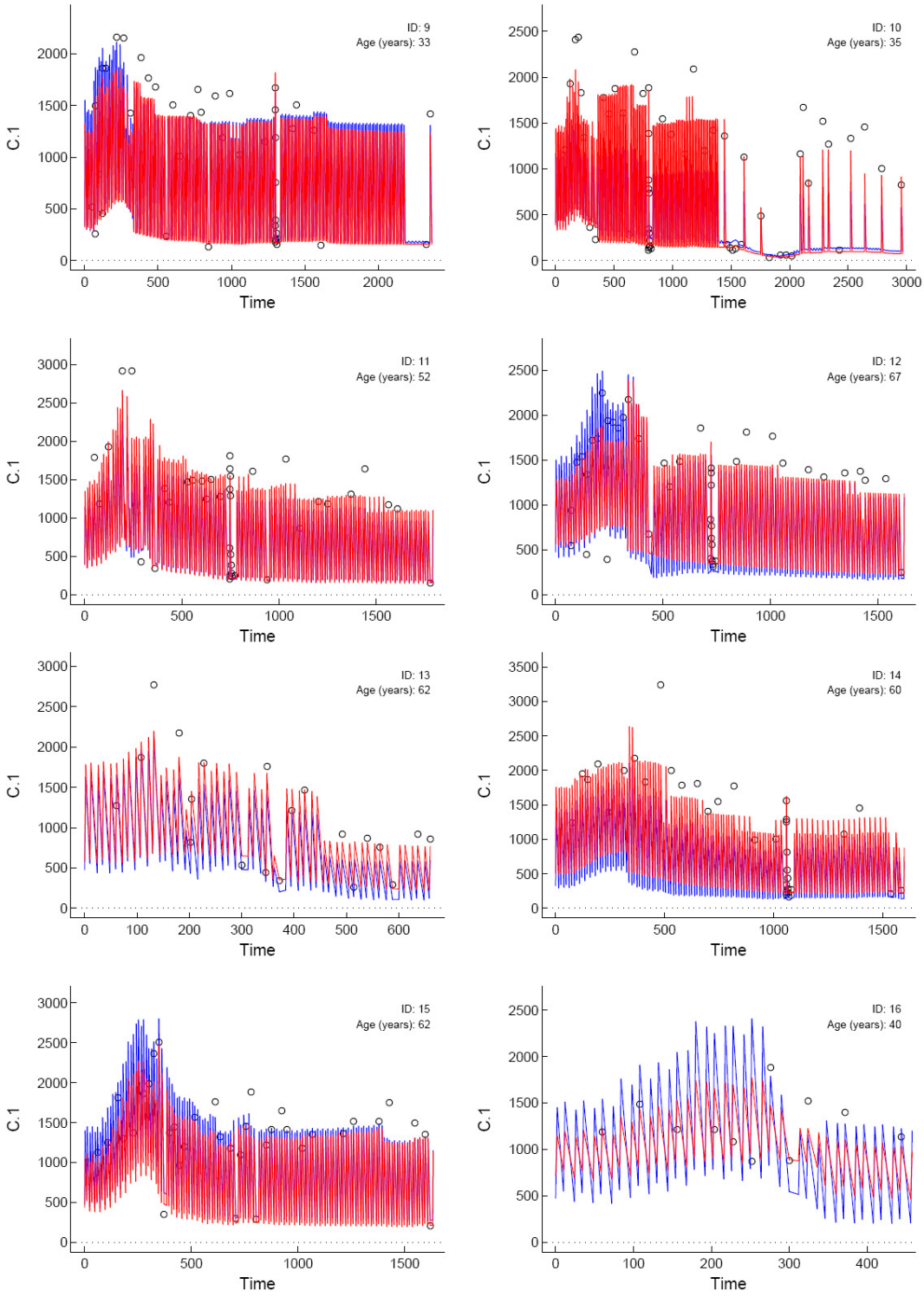
7.5 QUALITY OF FIT PLOT OF THE FINAL MODEL

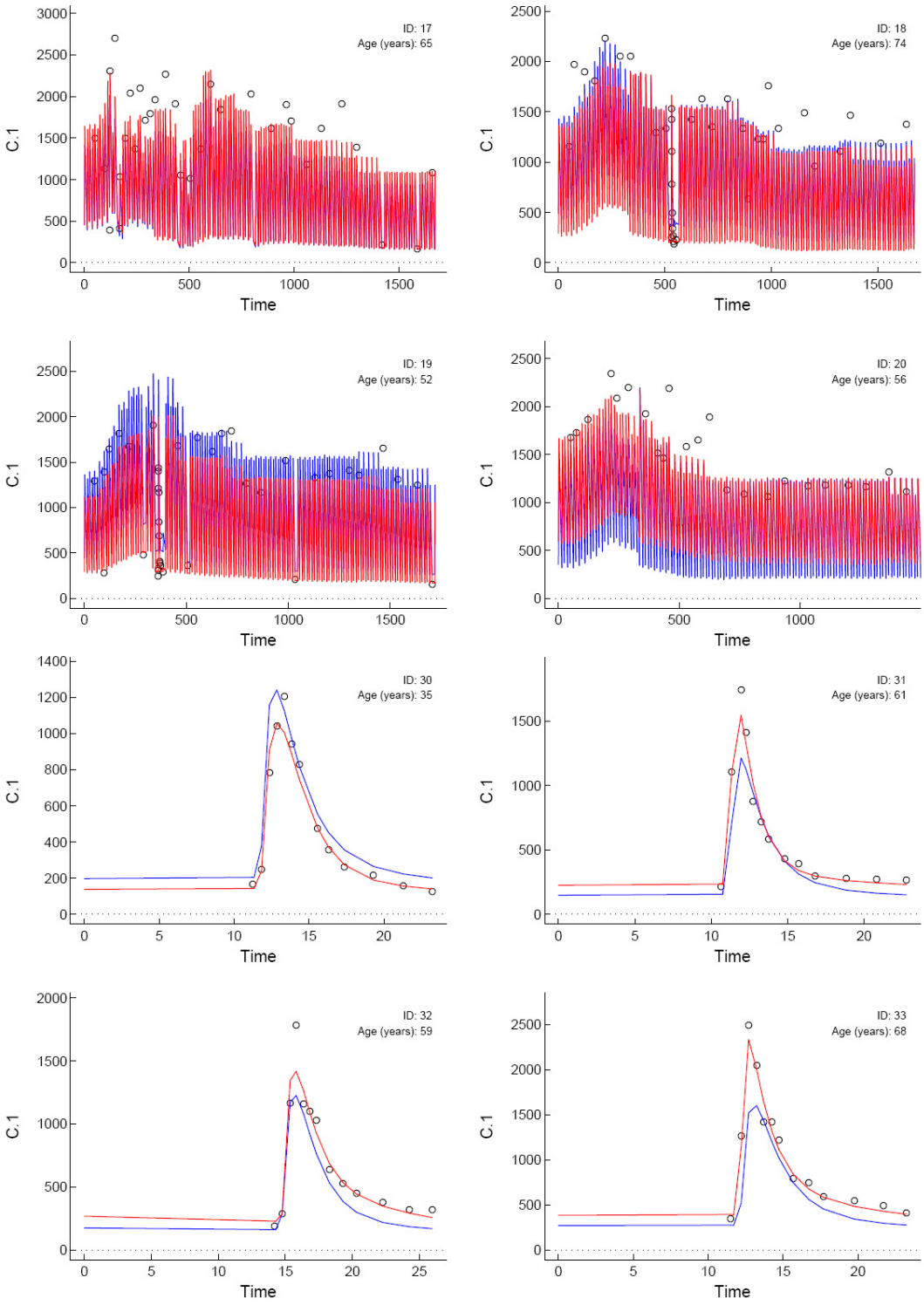


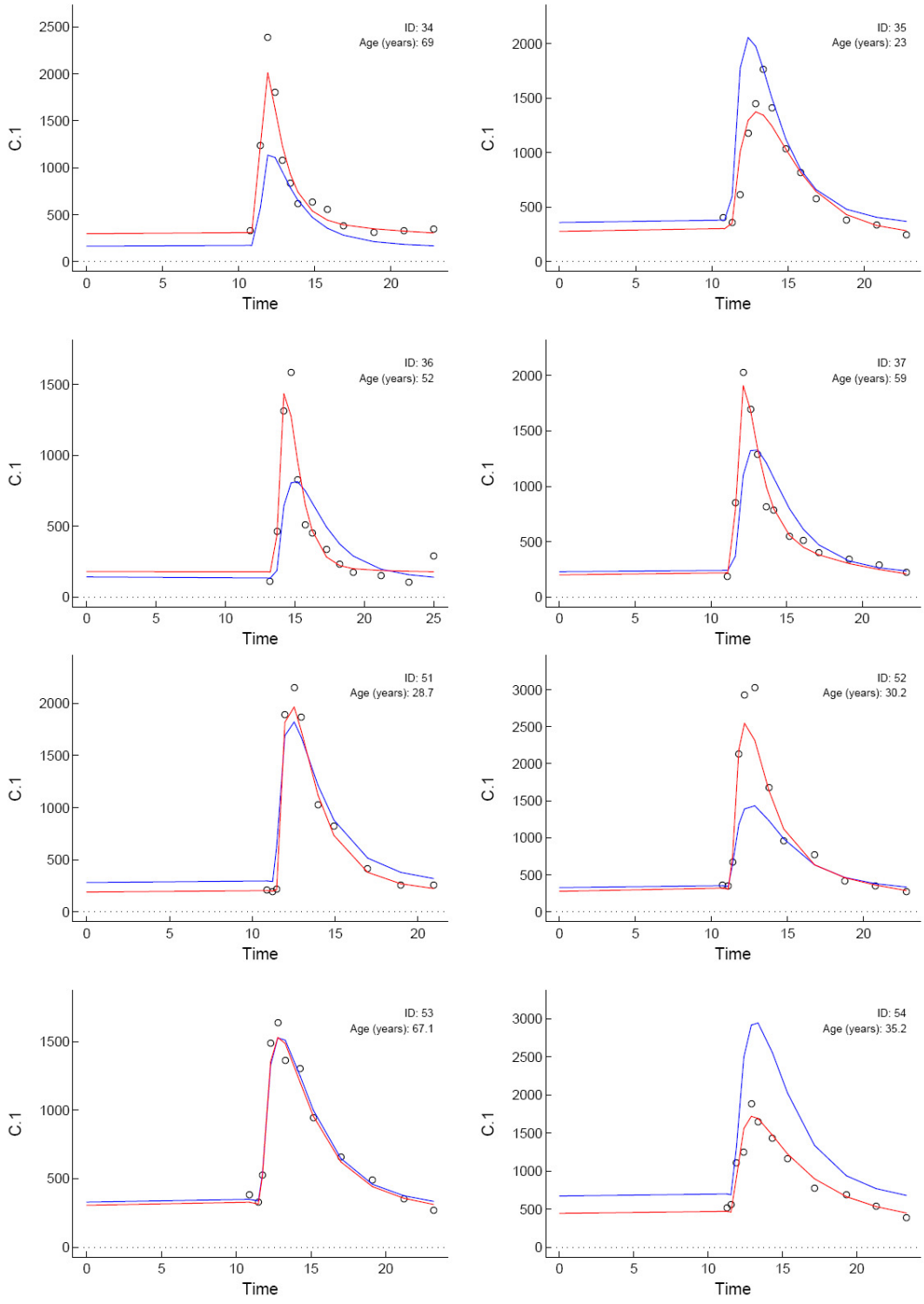
7.6 INDIVIDUAL FITS

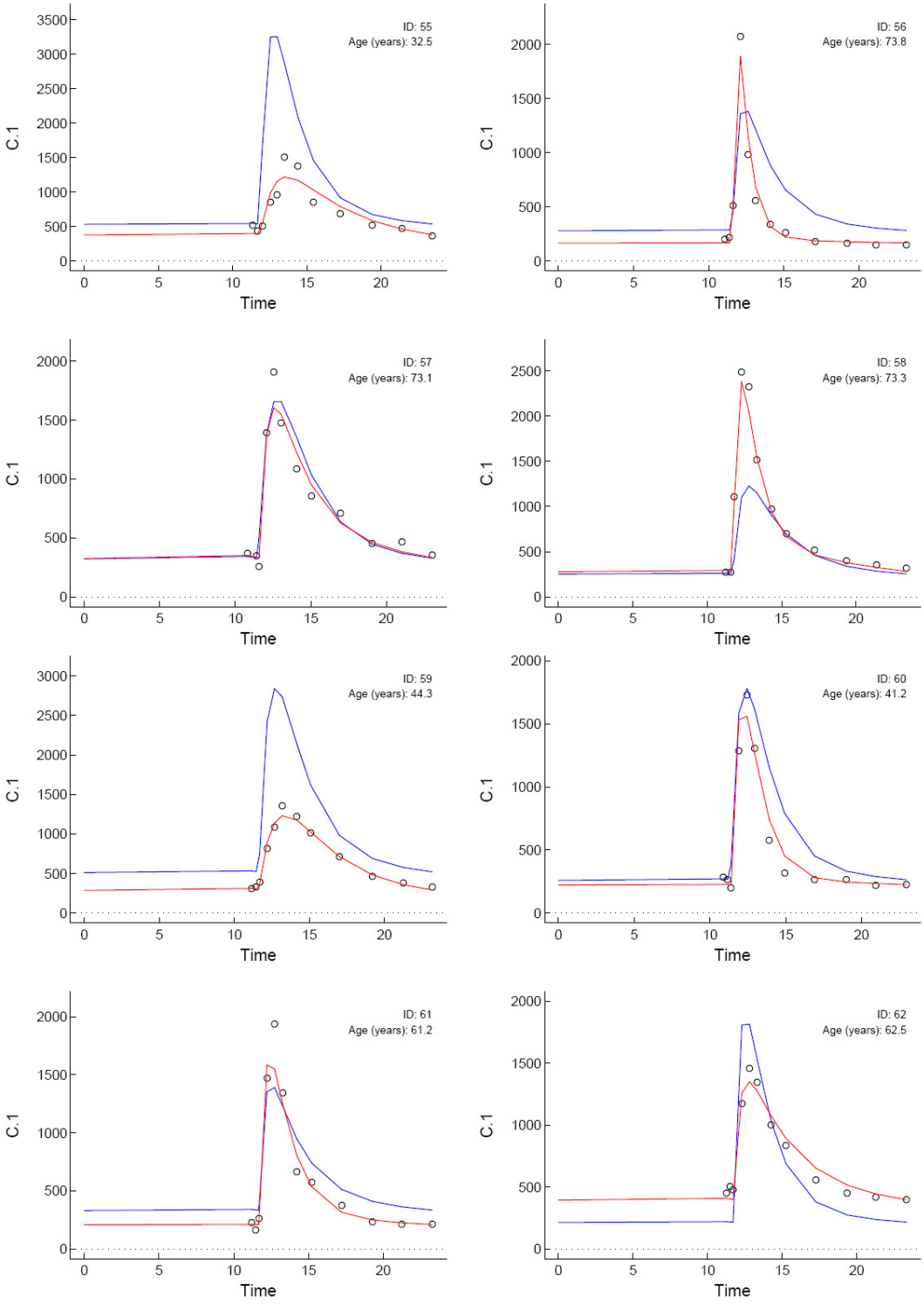
Circles: Observed concentrations; Red: Individual post hoc predicted concentrations; Blue: Population predicted concentrations

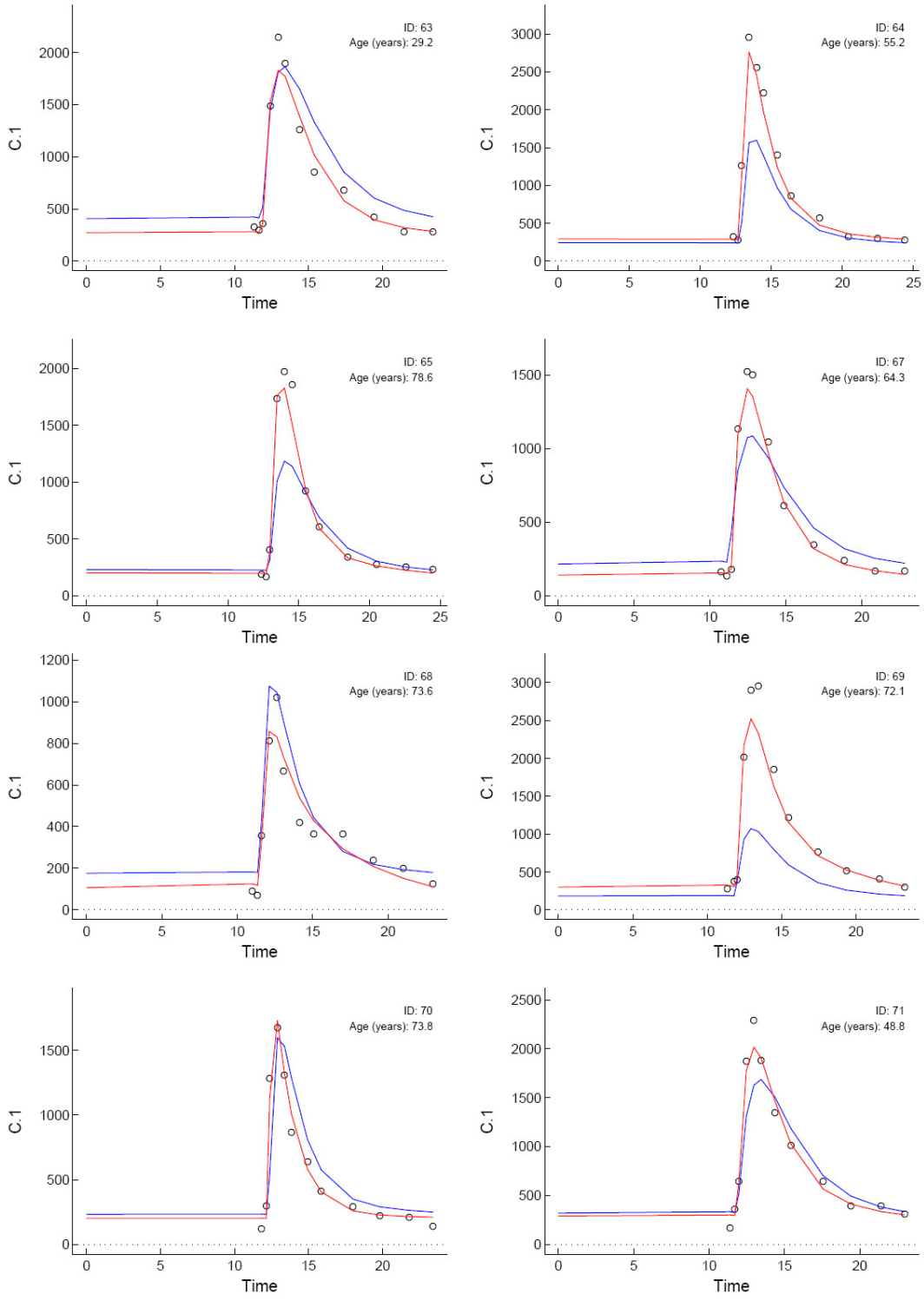


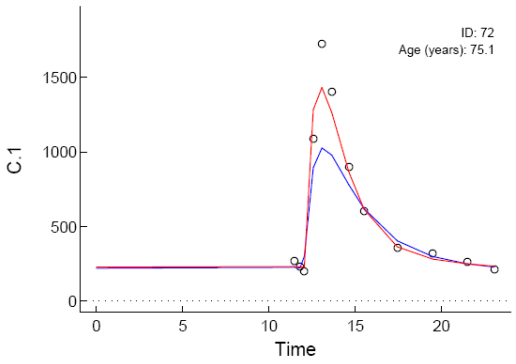

ID: 9
Age (years): 33
ID: 10
Age (years): 35
ID: 11
Age (years): 52
ID: 12
Age (years): 67
ID: 13
Age (years): 62
ID: 14
Age (years): 60
ID: 15
Age (years): 62
ID: 16
Age (years): 40
C.1
Time


ID: 17
Age (years): 65
ID: 18
Age (years): 74
ID: 19
Age (years): 52
ID: 20
Age (years): 56
ID: 30
Age (years): 35
ID: 31
Age (years): 61
ID: 32
Age (years): 59
ID: 33
Age (years): 68
C.1
Time


ID: 34
Age (years): 69
ID: 35
Age (years): 23
ID: 36
Age (years): 52
ID: 37
Age (years): 59
ID: 51
Age (years): 28.7
ID: 52
Age (years): 30.2
ID: 53
Age (years): 67.1
ID: 54
Age (years): 35.2
C.1
Time


ID: 55
Age (years): 32.5
ID: 56
Age (years): 73.8
ID: 57
Age (years): 73.1
ID: 58
Age (years): 73.3
ID: 59
Age (years): 44.3
ID: 60
Age (years): 41.2
ID: 61
Age (years): 61.2
ID: 62
Age (years): 62.5
C.1
Time


ID: 63
Age (years): 29.2
ID: 64
Age (years): 55.2
ID: 65
Age (years): 78.6
ID: 67
Age (years): 64.3
ID: 68
Age (years): 73.6
ID: 69
Age (years): 72.1
ID: 70
Age (years): 73.8
ID: 71
Age (years): 48.8
C.1
Time



7.7 JACKKNIFE RESULTS

PK parameters	Final model	Patient excluded							
		1	2	3	4	5	6	7	8
CL/F	26.1	25.6	23	26.1	26.1	26.1	25.9	25.4	23.2
V_C/F	77.7	78.8	78.2	78	77.9	77.8	77.9	77.9	78.2
Q/F	20.5	21.3	20.3	20.4	20.4	20.4	20.5	19.4	20.3
V_p/F	342	352	399	342	342	344	342	343	358
*k*a	1.88	1.9	1.83	1.86	1.86	1.9	1.79	1.74	1.7
ALAG	0.452	0.454	0.438	0.449	0.446	0.441	0.455	0.438	0.449

PK parameters	Patient excluded								
	9	10	11	12	13	14	15	16	17
CL/F	25.6	26.2	26.1	25.8	26.1	24.5	26.6	23.8	26.1
V_C/F	77.8	77.9	77.9	77.7	77.9	77.6	76.9	87.8	78.1
Q/F	21.1	20.4	20.4	21	20.6	17.7	20.3	17	20.4
V_p/F	344	342	342	350	342	322	343	399	342
*k*a	2.07	1.86	1.85	1.75	1.79	2.06	2.07	1.99	1.81
ALAG	0.454	0.451	0.448	0.448	0.459	0.447	0.434	0.451	0.445

PK parameters	Patient excluded								
	18	19	20	30	31	32	33	34	35
CL/F	26.1	28.2	25.6	24.1	23	26.1	24.9	26.3	26.2
V_C/F	77.9	77.5	79.8	78	77.3	77.9	77	78.6	77.8
Q/F	20.4	22.9	19.7	28.2	21.3	20.4	20.5	18.5	20.5
V_p/F	342	345	398	371	350	338	397	399	342
*k*a	1.81	1.87	1.44	1.62	1.84	1.86	1.82	1.64	1.93
ALAG	0.448	0.465	0.459	0.447	0.45	0.449	0.448	0.452	0.445

PK parameters	Patient excluded								
	36	**37**	**51**	**52**	**53**	**54**	**55**	**56**	**57**
CL/F	26	26.1	25.9	26.7	23.1	27	25.8	23	25.1
V_C/F	78.2	77.9	77.7	77.8	77.9	77.8	78	78.9	78.3
Q/F	20.4	20.4	20.5	20.5	18.9	21.3	20.4	17.5	21.8
V_p/F	342	342	326	356	344	320	339	341	346
*k*a	1.84	1.86	1.67	1.71	1.86	1.92	1.82	1.82	1.85
ALAG	0.443	0.447	0.449	0.443	0.452	0.443	0.455	0.455	0.446

PK parameters	Patient excluded								
	58	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**67**
CL/F	26.6	26.1	26.1	26.1	26	25.6	27.4	24.1	26.2
V_C/F	78.5	77.9	77.9	77.9	77.9	77.8	78.1	89.6	77.9
Q/F	17.5	20.6	20.4	20.6	20.4	19.6	19.6	20.5	20.7
V_p/F	342	342	342	345	343	353	344	399	342
*k*a	2.23	1.85	1.86	1.85	1.85	1.86	1.94	1.84	1.82
ALAG	0.464	0.449	0.446	0.453	0.447	0.449	0.446	0.447	0.461

PK parameters	Patient excluded				
	68	**69**	**70**	**71**	**72**
CL/F	26.1	23	26.1	27.8	23
V_C/F	77.9	94.8	77.9	75.1	77.9
Q/F	20.4	20.4	20.4	28.5	20.4
V_p/F	342	399	342	360	344
*k*a	1.86	1.99	1.86	2.12	2.79
ALAG	0.45	0.461	0.451	0.447	0.45